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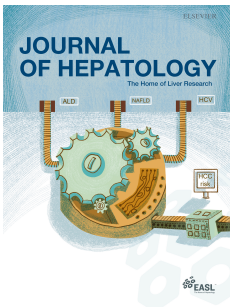

The role of Liver Sinusoidal Endothelial Cells in liver diseases: key players in health and pathology

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The role of Liver Sinusoidal Endothelial Cells in liver diseases: key players in health and pathology

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Key points

- LSECs are specialized liver cells that maintain hepatic homeostasis by controlling vascular tone, molecular exchange, immune regulation, and cellular crosstalk within liver sinusoids.
- LSEC dysfunction is common to all chronic liver diseases through a dedifferentiation process that includes capillarization (loss of fenestrae and formation of basement membrane), inflammatory activation, and gain of prothrombotic properties, serving as an early unifying mechanism across different liver pathologies.
- Insult-driven LSEC dysfunction promotes chronic altered biomechanical cues, which become key drivers of chronic liver disease progression and hepatic microcirculatory failure.
- LSECs play a key role in major liver diseases including MASLD/MASH, alcohol-associated liver disease, cirrhosis, acute liver injury, cholestatic diseases, and hepatocellular carcinoma, regulating immune responses by acting as antigen-presenting cells, maintaining tolerance in healthy conditions, but promoting inflammation and immune dysfunction during disease states.
- Advanced research tools are enabling new discoveries including single-cell RNA sequencing revealing LSEC heterogeneity, liver organoids, and liver-on-chip technologies for studying LSEC biology and interactions.
- Significant knowledge gaps remain particularly in understanding molecular mechanisms of LSEC dedifferentiation and translating preclinical findings to effective clinical therapies, highlighting the need for precision medicine approaches.
- Therapeutic targeting of LSECs shows promise with multiple molecular targets identified and innovative drug delivery approaches using nanotechnology being developed.

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Abstract

Liver sinusoidal endothelial cells (LSECs) are specialized endothelial cells that orchestrate the hepatic homeostasis within the liver sinusoid. Besides their key role in regulating intrahepatic vascular tone, trafficking and cell-crosstalk, their scavenging and immune-regulatory role makes them central in the development of any liver disease. LSECs dysfunction includes loss of fenestrae, inflammatory activation and the gain of vasoconstrictive and prothrombotic functions. Robust evidence demonstrated how preserving LSECs is crucial in pathological context, placing LSECs as the center for novel therapeutic and diagnostic strategies. Despite innovative tools supporting the dissection of LSECs heterogeneity, our understanding of LSECs pathobiology remains characterized by important gaps. This review highlights the recent discoveries as well as examines possible innovative therapeutic strategies targeting LSECs in liver disease.

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1. Introduction

Hepatic function depends on the hepatic sinusoid within which portal and arterial blood mix at low pressure and flow. Here, heterogenous population of endothelial cells, called liver sinusoidal endothelial cells (LSECs), act as primary gatekeepers with a unique and dynamic role [1]. LSECs present fenestrae and lack a basement membrane. Their selective permeability allows for a bidirectional exchange of soluble molecules and nanoparticles through the space of Disse. The fenestrae in LSECs are crucial for scavenging activity, enabling endocytosis. They also activate immune responses by presenting antigens and releasing cytokines and chemokine signals [2]. These activities give them an immune-regulatory role. LSECs dynamic role is exerted by regulating the contraction of the surrounding hepatic stellate cells (HSCs) via paracrine and angiocrine release of vasoactive molecules including nitric oxide (NO) [3, 4]. In addition, LSECs balance coagulation pathways by producing both procoagulant and anticoagulant factors, allowing the interaction of neutrophils and circulating platelets [5, 6].

This review focuses on the pivotal roles of LSECs in the pathogenesis and progression of chronic liver disease. It highlights recent discoveries in LSEC biology and identifies research gaps in understanding their complex functions. Additionally, the review examines emerging therapeutic strategies that target LSECs. By emphasizing the latest advances, it showcases the potential of LSEC-focused interventions to drive innovative therapies for liver diseases.

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2. LSECs in liver physiology: guardians of the hepatic microenvironment

2.1 Morphology and unique characteristics of LSECs

The unique morphology of LSECs, the presence of fenestrae and the lack of basal membrane is well known since 1970 [7]. Fenestrae, covering 2-20% of LSECs surface, are transmembrane pores between 100-200 nm of diameter grouped in sieve plates [8, 9]. The primary structure of fenestrae, the fenestrae-associated cytoskeletal ring (FACR) is composed of spectrin and actin proteins [10, 11]. The oxidation of thiol groups of spectrin regulates fenestration dynamics via protein disulfide isomerase (PDI) A1 [12].

Liver vasculature, including LSECs, has a substantial developmental contribution from the embryonic endocardium, with endocardial cells representing a key lineage source for hepatic endothelial populations [13]. Nevertheless, LSECs subsequently acquire highly heterogeneous phenotypes and specialized functions within the adult liver. Recent single-cell RNA sequencing (scRNAseq) revealed zonal heterogeneity among LSECs, with specific molecular and functional markers which confirm a very organized distribution of functional subtypes across the live lobule from periportal to pericentral zones **(Box 1)** [14-23]. However, nowadays the gold-standard to recognize LSECs is still the identification of fenestrae through scanning electron microscopy (SEM) [24, 25], atomic force microscopy (ATM) [26] or super-resolution optical microscopy [27].

Box 1. List of key LSECs-specific markers and their zonation.

Zone	Marker	Characteristics	Major Function
Z1	F8	↑ periportal	Coagulation factor
	PECAM1	↑ periportal	Regulator leukocyte trafficking
	vWF	↑ than mid	Hemostatic function marker
Z 2/3	CD32b	↑ mid-zone	Immune complex scavenging
	Lyve1	↑ mid-zone	Hyaluronan receptor, clearance
	Oit3	↑ mid-zone	Hepatic development, HCC, metabolism
	Stab2	↑ mid-zone	Scavenger receptor, endocytosis
	CD14	↑ zone 3	LPS receptor, immune response
	Flt4	↑ mid-zone	VEGF receptor, lymphatic growth
	Mrc1	All zones, ↑ in 2/3	Mannose receptor, endocytosis
All	Stab1	All zones	Scavenger receptor
	CLEC4G	All zones	Pathogen binding, immune regulation

2.2 Role of LSECs in liver homeostasis

LSECs, together with hepatic macrophages (HMs) and dendritic cells, represent the hepatic reticulo-endothelial system, contributing to clearance of systemic and portal blood [28]. LSECs act as antigen-presenting cells (APCs) through Stabilin 1 and 2 (Stab1 and 2) as scavenger receptors and the mannose receptor C-type 1 (Mrc1) [29, 30], among others. Moreover, LSEC are capable of priming naïve CD4 T cells through the antigen presentation of major histocompatibility complex (MHC) II [31, 32], and cross-presentation of soluble antigens on MHC-I to CD8 T cells [33], exerting the immunoregulatory role.

Due to their localization, LSECs regulate hepatic homeostasis through angiocrine signalling [34]. A key role of LSECs is to regulate HSCs quiescence and a physiological intrahepatic resistance via releasing of NO trough Krüppel-like factor 2 (KLF2)-dependent mechanism [35, 36]. Recently, the endothelial transcription factor (TF) *Gata4* has been found essential in maintaining HSCs and LSECs phenotype [37].

LSECs angiocrine signaling via cytokines and growth factors maintain and support hepatocyte regeneration and function [38]. Wingless (Wnt) proteins, such as Wnt2 and Wnt9b have been described as angiocrine mediator of liver regeneration through activation of the β–catenin pathway [39]. Angiocrine signaling is also regulated by cell crosstalk. Hepatocytes release vascular endothelial growth factor (VEGF), which after binding with LSECs receptors VEGFR-1 and -2, stimulates the release of hepatic growth factor (HGF) with a consequent acceleration of liver regeneration [40]. C-X-C chemokine receptor (CXCR)7 expression in LSECs promotes secretion of angiocrine factors like Inhibitor of DNA binding 1 (Id1), supporting liver regeneration via the VEGFR2-Id1 pathway after acute injury. In contrast, chronic injury increases CXCR4 over CXCR7, shifting LSECs toward a pro-fibrotic response [41].

Furthermore, LSECs maintain the homeostasis through autophagy and its impairment leads to dysfunctional endothelial phenotype and promotes liver disease [42].

2.3 LSECs in metabolism and detoxification

One of the main functions of LSECs is the scavenging of waste products through endocytosis. Low-density lipoproteins (LDL) are easily oxidized (oxLDL) promoting hepatic sinusoidal endothelial dysfunction as well as atherosclerosis development [43, 44]. Plasma mildly oxLDL molecules are scavenged by LSECs via the scavenger receptor Stab1 to clear atherogenic lipoproteins [45]. Moreover, the LSEC dysfunction observed at early stages of MAFLD occurs partly by oxLDL injury [46]. OxLDL reduces the expression of LSEC Sortilin, an LDL clearance receptor, which can be restored by inhibition of extracellular signal-regulated kinases (ERK) signalling pathway [47].

Recent studies showed that LSECs help regulate liver metabolic functions. Notably, LSEC-derived Wnt2 is essential for hepatocyte cholesterol uptake and its conversion to bile acids [48]. However, LSECs influence on bile acid and cholesterol pathways is not fully understood.

3. LSEC de-differentiation as a precursor to liver disease

3.1 Early endothelial dysfunction: a common pathway in liver diseases

Inflammatory activation. Biochemical insults from the sinusoidal lumen initially activate the hepatic endothelium in almost every liver disease, bridging altered metabolic, inflammatory, and fibrotic processes. Key hallmarks include the loss of fenestrae, development of basal membrane, increased oxidative stress, altered cell crosstalk, pro-inflammatory phenotype, and de-differentiation towards vascular unspecialized endothelial cells **(Figure 1A)** [2]. Moreover, LSECs clathrin-mediated endocytic activity becomes impaired, exacerbating the inflammatory response.

Capillarization. LSECs dedifferentiation in early stage is characterized by gradual but reversable reduction in number and size of fenestrae [49]. In cirrhosis, this progression is more severe in pericentral LSECs (zone 3), where cells show downregulation of LSECs specific markers, including LYVE1, CD32b, and Fms-related tyrosine kinase 4 (FLT4) [17]. The formation of a basal membrane is mainly due to deposition of collagen IV and laminin in the space of Disse [50]. This disrupts signaling pathways such as VEGF, Hedgehog, and bone morphogenetic protein (BMP)9, which are critical regulators of fenestration dynamics [51-53]. Then, the autophagic degradation of caveolin-1 triggers cytoskeleton remodeling, with pronounced effects in zone 3 in cirrhotic livers [17, 54]. This defenestration is exacerbated by inhibition of NO

bioavailability, combined with increased reactive oxygen species (ROS), to finally sustain capillarization (complete dedifferentiation). During capillarization in acute liver injury (ALI), LSECs develop a prothrombotic phenotype due to vasoconstriction, as recently reviewed [55]. These changes stimulate the release of interleukin (IL)-33 from LSECs, which induces neutrophil extracellular traps [56]. In chronic injury, additional alterations occur. The dysregulation of the Hedgehog gene family promotes fibrosis [57]. LSEC dedifferentiation due to activation of Notch signaling, accelerates liver fibrosis via impairment of endothelial nitric oxide synthase and soluble guanylate cyclase (eNOS-sGC) signaling, further compromising hepatocyte function [58]. Moreover, semaphorin 3A (SEM3A) upregulation is directly involved in defenestration of LSECs by reducing the fenestrae frequency and porosity in steatotic mice via Neuropilin-1–LIM domain kinase 1 (NRP1-LIMK1) cascade. Specific deletion of SEMA3A reduced hepatic steatosis and liver fibrosis in animal models for obesity, type 2 diabetes and Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) [59]. Reversing LSEC capillarization in the late stages of MASH may be experimentally challenging and less effective than early disease [49].

Single-cell studies, have identified scar-associated endothelial populations in human liver fibrosis, marked by PLVAP and ACKR1 positivity, which appear in fibrotic septae and demonstrate distinct pro-fibrogenic features [21]. However, it remains debated whether these cells constitute a new endothelial population or arise from dedifferentiation of resident LSECs. As previously discussed, defenestration and phenotypic change of LSECs during capillarization could result from local dedifferentiation or from recruitment and engraftment of bone marrow–derived sinusoidal progenitor cells (BM SPCs) that replace lost LSECs and contribute to vascular remodeling in injury and regeneration [60].

Immune activation. Decreased NO production across all lobule zones, along with ROS accumulation and extracellular matrix (ECM) component deposition, collectively drive the impairment of LSECs barrier function. The secretion of cytokines and chemokines, including tumor necrosis factor-alpha (TNF-α), IL-6, and monocyte chemoattractant protein-1 (MCP-1/CCL2), initiates the inflammatory response, as previously described [2]. Endothelial inflammation worsens hepatocyte injury and activates HSCs, which leads to increased intrahepatic vascular resistance. The severity of LSEC dysfunction is closely linked to hepatocyte damage and the stage of

fibrosis, as observed in hepatitis C virus (HCV)-related chronic hepatitis [61]. Dysfunctional LSECs with loss of Gata4-dependent signals upregulate membrane vascular cell adhesion molecule (VCAM1) and platelet endothelial cell adhesion molecule (PECAM-1/CD31) [37]. CD34 upregulation is observed in all zones, with a peak in zone 3 [17]. Changes in antigen presentation and T cell recruitment have been noted in early MASH and fibrosis stage [62, 63]. The recruitment of immune cells by LSECs is a critical milestone in chronic liver disease (CLD) progression.

Biomechanical stress dysregulation. Mechanical stimuli are master regulators of LSEC phenotype [64]. Chronic biomechanical alterations regulate nuclear trafficking and the expression of biomechanically modulated molecular factors (BMMFs) [65]. Increased mechanical stretching causes downregulation of protective endothelial specific-TFs like Erythroblast Transformation Specific (ETS)-related gene (ERG), an early marker and endothelial-specific regulator of canonical transforming growth factor beta (TGFβ)/BMP signaling [66, 67]. ERG is an important regulator of vascular homeostasis maintenance due to its anti-inflammatory and anti-thrombotic functions, responsible for preventing endothelial-to-mesenchymal transition by impeding SMAD3-mediated TGF-β signaling [68].

Dysregulated Rho-GTPases and Rho-associated protein kinase (ROCK) activity enhance cytoskeletal contraction, increasing intrahepatic vascular resistance [69, 70]. Increased matrix stiffness activates profibrotic TFs, such as Yes-associated protein (YAP)/Transcriptional coactivator with PDZ-binding motif (TAZ) and (myocardin-related transcription factor A) MRTF-A [71]. Finally, intracellular stiffness mediators, BMMFs, are directly linked to the release of pro-inflammatory cytokines [72]. LSEC dysfunction also affects shear-stress responses. Oxidative stress induced by reduced NO impairs the regulation of sinusoidal tone, driving HSC contraction and raising portal pressure. Shear stress modulates TFs responsible for NO release and LSEC functions, such as KLF2, KLF4, and the (Activator Protein (AP-1) complex (including bZIP family members (FBJ murine osteosarcoma viral oncogene homolog) FOS, FOSb, JUN, and JUNb) [73, 74]. These factors are often downregulated in cirrhosis [17]. Increased hydrodynamic pressure further disrupts LSEC function. Chromobox (CBX)7, a pressure-sensitive TF, modulates E-cadherin and serine peptidase inhibitor Kazal type 1 (SPINK1) under high pressure. Both proteins represent potential blood biomarkers for portal hypertension [75]. Moreover, the epigenetic changes may

stabilize a dysfunctional phenotype of non-parenchymal cells contributing to CLD progression as demonstrated in other cell types [76].

In MASLD, the liver sinusoid undergoes structural changes altering hepatic cell mechanotransduction. Hepatocytes ballooning, a key MALSD feature, promotes intracellular compression, increase hepatocyte diameter which reduces the space of Disse and altering hepatic cells cytoskeleton dynamics [77]. Recently, a process known as single-cell steatonecrosis has been identified via electron microscopy. This is driven by fat extrusion from dying hepatocytes that results in the formation of lipid emboli in the lumen of sinusoids [78]. In early MASLD, LSECs reduce the release of vasodilators in response to increased shear stress [79]. Capillarization of LSECs can occur at early stages of MASLD, even before steatosis develops, or HMs and HSCs are activated. A recent study showed that SEMA3A interferes with VEGF signaling in MASLD in both human and mouse LSECs, leading to significant defenestration [59].

3.2 Oxidative stress and endothelial damage

Unlike hepatocytes, which require stable oxygenation to function, LSECs show a low abundance of mitochondria defining a unique metabolic profile that changes across the lobule zonation [80, 81]. However, LSECs primarily generate ROS through alternative enzymatic pathways rather than Nicotinamide adenine dinucleotide phosphate oxidases (NOX)/Nicotinamide adenine dinucleotide phosphate (NADPH) [82]. As previously described, xanthine oxidase (XO) is a principal source of ROS on dysfunctional LSECs, particularly under conditions of oxidative stress or altered shear stress [83]. XO increases the production of uric acid and form superoxide anion and H_2O_2as sub-products. Other contributors to ROS in LSECs may include peroxisomal oxidases and cytochrome P450 enzymes [84]. Moreover, LSECs may be more susceptible to ROS due to the lack H_2O_2-detoxifying enzymes [85]. Accumulation of ROS in LSECs leads to functional impairment, including defenestration, reduced endocytic and scavenging capacity, and disruption of cytoskeletal integrity. These oxidative changes compromise the LSEC barrier and promote capillarization, which in turn fails to maintain HSC quiescence and facilitates fibrogenic signaling. Key molecular pathways affected by oxidative stress in LSECs include dysregulation of angiogenic signals such as VEGF and eNOS, further contributing to hepatic microvascular dysfunction and progression of liver disease [86, 87].

3.3 Senescence of LSECs in aging and liver disease

As individuals age, the liver undergoes degenerative changes as extensively reviewed [88-90]. The growing elderly population has created increased demand for liver transplants while simultaneously expanding the pool of aged donors and recipients [91]. This demographic shift has intensified focus on understanding liver microcirculatory dysfunction in aging, a critical factor that could improve donor-recipient matching and transplant outcomes.

Changes include hepatocyte polyploidization, enlargement, and senescence, along with reduced nutrient extraction capacity due to decreased blood flow and impaired signaling pathways (mTOR, sirtuins, AMPK, insulin/IGF). The aging liver also experiences mitochondrial dysfunction, compromised proteostasis, reduced ischemia tolerance, and slower drug metabolism [92].

Aged LSECs show pseudocapillarization (fewer fenestrae, thicker basement membranes, impaired perfusion) resembling capillaries [93, 94]. Aged CCl_4 cirrhotic rats display capillarization, reduced vasoprotective factors, and higher portal pressure [24]. Simvastatin and sirtuin (SIRT)1 activation improve vasodilation, re-fenestration, and matrix reduction [24, 95]. SIRT1, via p53 deacetylation or SRT1720, reduces senescence and inflammation in CCl_4 models [96, 97]. Lower Stab2 reduces endocytosis and waste clearance [98, 99]. Decreased VEGFR2 and NO increase portal pressure in aged LSECs [92, 100]. Aging induced-profibrotic pathways promote LSECs senescence, increasing senescence-associated secretory phenotype (SASP), p16 expression, oxidative stress, TNF-α, IL-1, and IL-6, which supports leukocytes adhesion and chronic inflammation [92, 101, 102].

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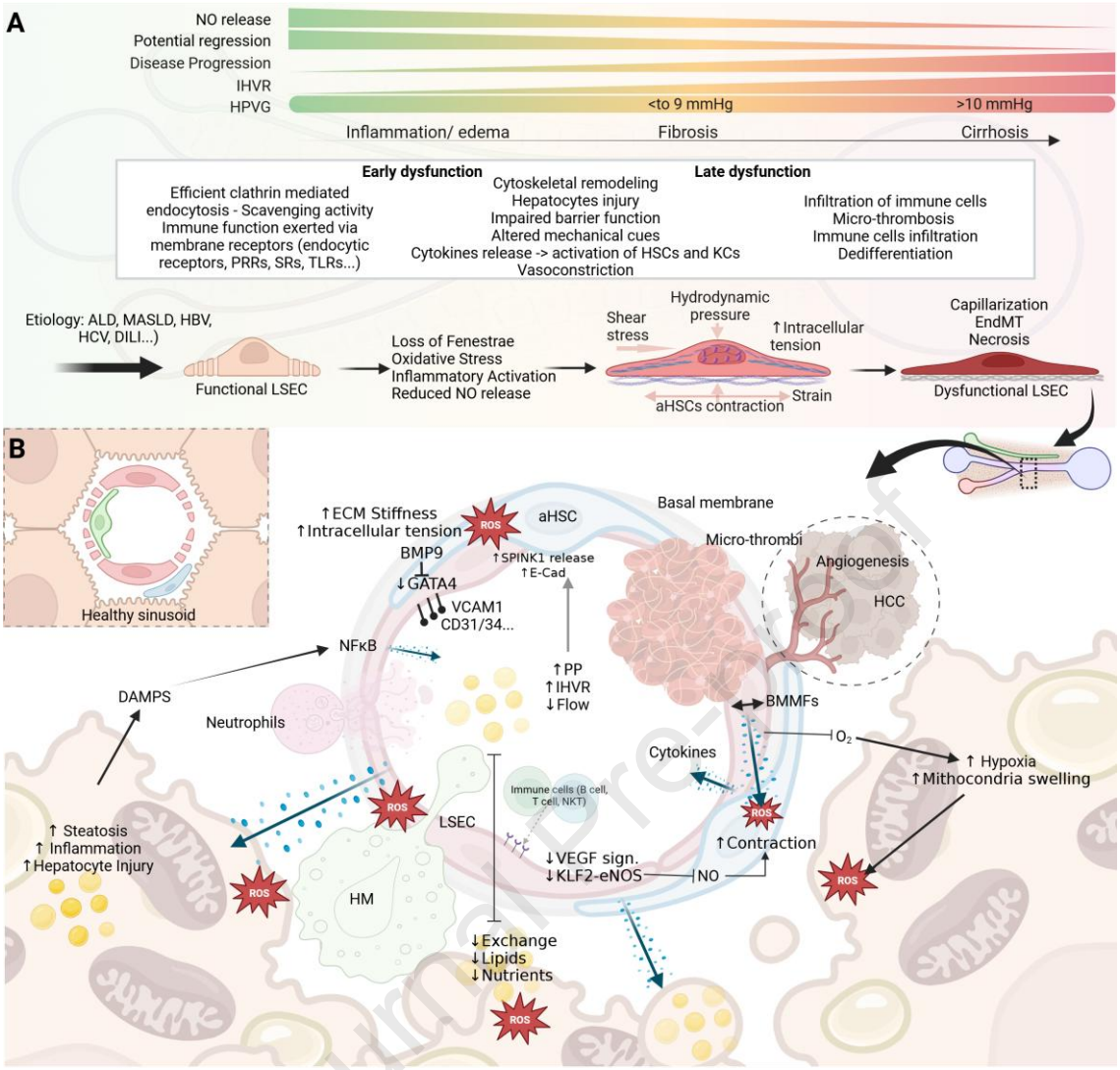

Figure 1. LSECs dedifferentiation in chronic liver disease. A) Progression of chronic liver disease is accompanied and aggravated by the sinusoidal endothelial dysfunction. Endothelial dysfunction results in increased hepatic vascular resistance and portal hypertension. **B)** Inside the sinusoidal space, LSECs crosstalk orchestrate hepatic lobule molecular trafficking and function. Due to persistent insults from the portal and systemic circulation LSECs crosstalk is altered with the release of proinflammatory factors as well as with reduced soluble factors like NO that are involved in maintenance of non-parenchymal cell phenotypes. Further activation of HSCs stimulates their contraction increasing the hepatic resistance and a pro-thrombotic environment. Abbreviations: aHSC (activated hepatic stellate cell), ALD (alcoholic liver disease), BMMFs (biomechanically modulated molecular factors), BMP9 (bone morphogenetic protein 9), CD31/34 (cluster of differentiation 31/34), DAMPS (damage-associated molecular patterns), DILI (drug-induced liver injury), E-Cad (E-cadherin), ECM (extracellular matrix), eNOS (endothelial nitric oxide synthase), EndMT (endothelial-to-mesenchymal transition), GATA4 (GATA binding protein 4), HCC (hepatocellular carcinoma), HBV (hepatitis B virus), HCV (hepatitis C virus), HSC (hepatic stellate cell), HVPG (hepatic venous pressure gradient), IL (interleukin), Indo (indomethacin), IVHR (intrahepatic vascular resistance), HMs (Hepatic macrophages), KLF2 (Krüppel-like factor 2), LSEC (liver sinusoidal endothelial cell), MASLD (metabolic dysfunction-associated steatotic liver disease), NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells), NO (nitric oxide), O_2 (oxygen), PRRs (pattern recognition receptors), ROS (reactive oxygen species), SPINK1 (serine peptidase inhibitor Kazal type 1), SRs (scavenger receptors), TLRs (toll-like receptors), VCAM1 (vascular cell adhesion molecule 1), VEGF (vascular endothelial growth factor).

4. Role of LSECs in liver diseases

4.1 LSEC in MASLD

MASLD is currently a prevalent liver condition [103]. In the early stages, LSECs dysfunction is led by factors such as lipotoxicity, insulin resistance, and gut microbiota dysbiosis, altering the angiocrine signaling [104]. Secondary events are immune system and HSCs activation [105]. Lipotoxicity from free fatty acids accumulation impair LSECs phenotype [106], while insulin resistance exacerbates inflammation via cytokine release and reduction of NO availability [107, 108]. Moreover, gut microbiota alterations aggravate inflammation via endotoxin translocation [109, 110].

LSECs regulate lipid exchange with hepatocytes through fenestrae and endocytosis [111]. In MASLD, reduced sinusoidal permeability impairs LDL secretion, contributing to hyperlipidemia [79, 112]. LSEC dysfunction also fails to maintain HSC quiescence, while hepatocellular lipotoxicity induces mitochondrial dysfunction, endoplasmic reticulum stress, and lipo-apoptosis [113]. Hyperlipidemia-driven oxidative stress perpetuates inflammation [114]. LSEC defenestration, alongside VEGF and chemokine release from HSCs, promotes aberrant vascular remodeling. Capillarized LSECs, as a shared mechanism in CLD, secrete pro-fibrogenic cytokines (TGF-β1, platelet-derived growth factor (PDGF), ILs, TNFα, VEGF), driving HSC transdifferentiation into myofibroblasts and fibrosis progression [79]. In this context, activation of Peroxisome Proliferator Activated Receptor Alpha (PPARα)/Sterol regulatory element binding proteins (SREBP1c) in hepatocytes further promote lipid accumulation [115]. **Box 2** summarizes the key angiocrine events occurring during MASLD-MASH progression in LSECs and their crosstalk with other sinusoidal cells.

BOX 2 Main events in LSECs crosstalk with hepatocytes, HMs and HSCs in MASLD and MASH

Crosstalk	MASLD (early disease)	MASH (advanced disease)
LSECs-Hepatocyte	Impaired lipid transport; reduced fenestration leading to mild hepatic steatosis via PPARγ/SEBP1c; eNOS-NO decrease, affecting glucose metabolism.	Severe endothelial dysfunction; capillarization and hypoxia promoting hepatocyte apoptosis and oxidative stress. Hepatocytes release DAMPs in response to lipotoxicity aggravating LSECs inflammatory response via NF-kB and JNK path. Reduced hepatocyte-VEGF release activates LSECs which in turn express ET-1 further activating HSCs
LSECs-HMs	Mild HMs activation due to LPS via TLR4 activation; mild cytokine release (IL-1β, IL-6); homeostatic endotoxin clearance by LSECs (Stab-1/2).	Chronic HMs activation; sustained cytokine storm (TNF-α, IL-1β, IL-6) driving hepatocyte death and inflammation. LSECs upregulation of VCAM1 and ICAM-1 promote immune cell adhesion and circulating monocytes infiltration. TGF-β1 from HMs enhance LSECs dysfunction.
LSECs-HSCs	LSECs maintain VEGF secretion, preventing HSC activation and ECM deposition.	Pro-fibrogenic signaling; LSECs increased cytokine and reduced NO release fail maintaining HSCs quiescence inducing transdifferentiation into myofibroblasts and fibrosis progression.

4.2 LSECs in Alcohol-associated Liver Disease

Alcohol-associated liver disease (ALD) is driven by inflammation, with gut permeability and cell death playing key roles [116]. ALD progresses from steatosis to fibrosis, cirrhosis, with acute alcohol-associated hepatitis arising at any of the previously mentioned stages. From one side, chronic alcohol assumption induces leaky gut, where pathogens, bacterial endotoxins and lipopolysaccharide (LPS) translocate into the circulation and reach the liver through the portal vein, provoking the activation of macrophages (e.g., HMs). Activated HMs initiate a pro-inflammatory response via release cytokines like IL-6, TNF-α and IL1-β. This results in the activation of LSEC with the consequent described effect on immune cells, further sustaining this inflammatory response.

On the other side, the direct effect of alcohol metabolism in LSECs, primarily through CYP2E1, leads to excessive production of acetaldehyde and acetyl-CoA, which promote oxidative stress, cellular DNA and protein damage and protein acetylation respectively. This modification affects heat shock protein 90 (Hsp90), weakening its interaction with eNOS and reducing NO production, a key factor in maintaining endothelial function [117]. Moreover, Yang et al., showed that alcohol further increases CYP2E1 expression, which amplifies oxidative stress and accelerates Hsp90

acetylation, disrupting its role in stabilizing proteins and compromising endothelial phenotype [118]. Restoring histone deacetylase (HDAC)6 activity in liver endothelial cells, which reverses Hsp90 acetylation, improves Hsp90-eNOS interaction, and helps protect against alcohol-induced LSEC dysfunction and liver injury [118].

Beyond this, alcohol exposure upregulates pro-inflammatory genes in LSECs, such as CXCL1, hypoxia-inducible factor 1 alpha (HIF1α), and endothelin-1 (ET-1), promoting a vasoconstrictive and pro-inflammatory microenvironment [119]. In addition to restoring NO production, HDAC6 overexpression may ameliorates LSEC dysfunction by reducing HIF1α activity as in other cell types [120].

Consequent to LSECs hyperactivation, upregulation of adhesion molecules such as VCAM-1, ICAM-1, and CD146, facilitates immune cell infiltration into the liver, promoting fibrosis in synergia with the leaky gut effect [121]. The release of soluble forms of these adhesion molecules into circulation correlates with ALD severity and has been suggested as a potential biomarker for disease progression [122, 123]. Several chemokines such as CXCL1, CXCL6, and CXCL8 has been shown to be upregulated via TNF-α/NF-κB signaling, further recruiting neutrophils and promoting hepatotoxic inflammation in alcohol-associated hepatitis [124]. The reticuloendothelial system, comprising HMs and LSECs, is overactivated in severe ALD, with elevated markers of endothelial activation, such as von Willebrand factor (vWF), correlating with mortality [125]. IL-6 signaling through STAT3 in LSECs is thought to be protective in conventional signaling but detrimental via trans-signaling pathways, highlighting the complexity of inflammatory regulation in ALD [126, 127]. See **Box 3** for concise angiocrine events occurring during ALD progression.

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BOX 3 Main events in LSECs crosstalk with hepatocytes, HMs and HSCs in ALD

Crosstalk	ALD (early disease)	ALD (advanced disease)
LSECs-Hepatocyte	Alcohol metabolites induce DNA and protein damage, oxidative stress, reducing LSECs fenestration and NO synthesis via eNOS suppression. Impaired VEGF signaling disrupts hepatocyte-LSEC metabolic coordination.	Severe capillarization exacerbates hypoxia, promoting hepatocyte necrosis/pyroptosis. DAMPs from necrotic hepatocytes drive LSECs inflammation via NF-κB.
LSECs-HMs	Gut-derived LPS activates HMs via TLR4, releasing IL-1β/IL-6. LSECs endotoxin clearance (Stab-1/2) becomes impaired.	Chronic HM activation (M1 polarization) sustains TNF-α/IL-1β release, amplifying hepatocyte apoptosis and LSECs dysfunction. Endothelial VCAM-1/ICAM-1 upregulation promotes leukocyte adhesion.
LSECs-HSCs	LPS/TLR4 activation in LSECs stimulates PDGF/TGF-β release, initiating HSCs activation. Reduced NO from capillarized LSECs fails to maintain HSCs quiescence.	Sustained TGF-β/PDGF signaling drives HSC transdifferentiation into collagen-producing myofibroblasts. Angiocrine dysregulation (e.g., CXCRs imbalance) perpetuates fibrosis.

4.3 LSECs in liver fibrosis progression and development of cirrhosis

Advanced chronic liver disease (CLD) is characterized by long-standing fibrosis and inflammation where capillarized LSECs play a central role in both processes and in controlling the vascular tone (**Figure 1B**) [128, 129]. The angiocrine signalling of LSECs is key in the activation of HSCs. LSECs maintains HSC quiescence phenotype via the endothelial vasodilator NO [3, 52]. This mechanism is mediated by the translocation to the nucleus of the vasoprotector TF KLF2 [35, 36]. Statins improve hepatic endothelial dysfunction through activation of KLF2-NO pathway which subsequently alleviates liver fibrosis and portal hypertension in cirrhosis [35, 130]. Moreover, in this context the disruption of LSEC autophagy drives endothelial dysfunction, amplifies hepatic fibrosis. Autophagy synergically cross-talks with the transcription factor KLF2 in endothelial cells, to modulate endothelial phenotype and survival [131].

Reduced eNOS-dependent NO, regulated by RAC-alpha serine/threonine-protein kinase (AKT), is a hallmark of LSECs dysfunction and promotes HSCs activation [3, 132]. Pathways involving CXCR4/CXCR7 [133], Notch1/Piezo1 [134], NFκB [135, 136], GATA4 [37], and KLF2 [36] dictate LSEC fate. Therefore, preclinical studies suggest that treatments targeting NO signalling pathway ameliorate LSECs dysfunction which paracrinally reduces HSC activation, portal pressure, and liver

fibrosis [137-141]. Mechanical forces exacerbate dysfunction via sustained intracellular tension [64, 72]. Emerging studies continue to demonstrate the impact of angiocrine signals on HSC activation, suggesting new potential therapeutic strategies for the treatment of hepatic fibrosis. **Box 4** depicts the main sinusoidal crosstalk events occurring during CLD development.

On the other hand, it is well described that a sub-population of dedifferentiated LSECs undergo endothelial mesenchymal transition (EndMT) transforming into LSECs-myofibroblast as in response to chronic liver injury **(Figure 2)**. Specifically, EndMT is a complex biological process where endothelial cells progressively acquire a mesenchymal phenotype, characterized by morphological changes, increased motility, loss of endothelial cells-specific markers, and the initiation of mesenchymal-specific gene expression, including fibrillar collagens. Transitioned LSECs lose endothelial markers such as vWF and VE-cadherin while exhibiting increased levels of mesenchymal markers like α-SMA, vimentin, and collagens type I and III among others [142-144]. Single-cell RNA sequencing analysis of non-parenchymal cells from fibrotic mice demonstrated that LSECs achieve mesenchymal profile compared to healthy mice [144]. A cluster described as myofibroblast also expresses specific LSEC markers such as LYVE-1, Stab2 and Flt4. Additionally, different subtypes of LSECs identified along the hepatic zonation axis expressed mesenchymal or ECM-related markers with a downregulation of LSEC-associated genes. Interestingly, when LSEC capillarization was blocked by a specific activator of NO donor (YC-1), the EndMT process was abrogated. In this context, a recent study has described miR-27b-3p as an upstream regulator of EndMT in cirrhotic LSECs. Indeed, restoration of miR-27b-3p expression in cirrhotic LSECs using nanotechnology ameliorates their mesenchymal-like phenotype by recovering their endothelial phenotype characteristic of healthy LSECs, suggesting an alleviation of the EndMT process [145].

Furthermore, in ACLD several biomechanical alterations contribute and perpetuate portal hypertension [64]. Additionally, changes in liver stiffness by the increase of the ECM deposition, is also detected by LSECs inducing deterioration in their phenotype [65]. Recently, collagen IV secreted by LSECs has been described as an important contributor to their capillarization by formation of basement membrane. Specifically, mice with a LSEC-specific deletion of CoIVα1 showed reduced Col4 deposition in the

liver sinusoids with an amelioration in sinusoidal permeability and reduced portal pressure [146].

BOX 4 Main events in LSECs crosstalk with hepatocytes, HMs and HSCs in fibrosis and ACLD

Crosstalk	Fibrosis (early disease)	Cirrhosis (ACLD)
LSECs-Hepatocyte	Hepatocyte apoptosis releases DAMPs, inducing LSECs inflammation.	Senescent hepatocytes upregulate SASP (senescence-associated secretory phenotype), exacerbating LSECs capillarization. Hypoxia promotes hepatocyte derived DAMPs perpetuating LSECs dysfunction. Hepatocyte EVs contribute to LSECs dysfunction.
LSECs-HMs	M2 macrophages secrete IL-10/TGF-β, promoting HSC activation.	Macrophage-derived galectin-3 and TGF-β drive sustained HSC activation and collagen cross-linking.
LSECs-HSCs	Capillarization reduces NO production, removing HSC quiescence signals. LSEC-derived PDGF/TGF-β activates HSCs.	LSEC-derived thromboxane A2, leukotrienes and endothelin-1 promote HSC contractility, increasing portal pressure. CXCR4 dominance over CXCR7 shifts signaling toward fibrogenesis. Endothelial EVs further activate HSCs.

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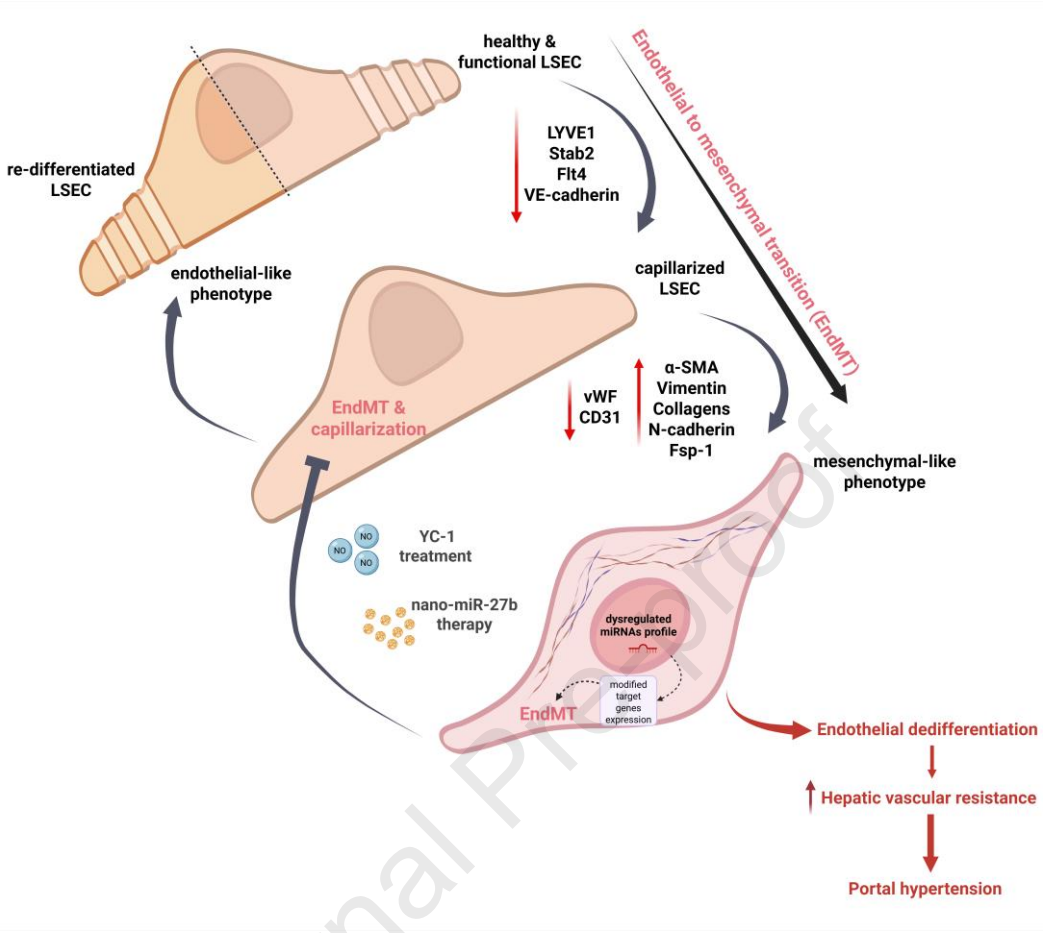

Figure 2. Endothelial to mesenchymal transition process of LSECs. Under liver injury, healthy LSECs "capillarize" losing their characteristic and unique phenotype including the endothelial markers LYVE-1, Stab2, Flt4 and VE-cadherin. When liver injury persists, a subpopulation of LSECs undergo endothelial to mesenchymal transition process (EndMT) acquiring mesenchymal-like phenotype with increased expression of mesenchymal molecules such as α-SMA, vimentin, collagens (type I and III), N-cadherin and FSP-1, and reduced endothelial markers like vWF and CD31 and dysregulation of miRNAs profile. Altogether these processes promote endothelial dedifferentiation and dysfunction, leading to the increase of hepatic vascular resistance and the development of portal hypertension. Interestingly, treatments with nitric oxide donors (YC-1) or miRNAs (miR-27b-3p) alleviate the EndMT through the recovery of the specific endothelial phenotype. Abbreviations: α-SMA (alpha-smooth muscle actin), CD31 (cluster of differentiation 31), Flt4 (fms-related tyrosine kinase 4), Fsp-1 (fibroblast-specific protein 1), LYVE1 (lymphatic vessel endothelial hyaluronan receptor 1), miRNA (microRNA), N-cadherin (neural cadherin), NO (nitric oxide), Stab2 (stabilin-2), VE-cadherin (vascular endothelial cadherin), vimentin (vimentin), vWF (von Willebrand factor), YC-1 (3-(5'-hydroxymethyl-2'-furyl)-1-benzyl indazole).

4.4 LSECs in Acute Liver Injury and Acute-on-Chronic Liver Failure

Hepatic endothelium regeneration is key for a functional restoration of the liver to maintain homeostasis and in acute liver injury [147]. Loss of LSEC integrity goes along with liver failure [148-150]. Liver regeneration post-two-thirds partial hepatectomy (PHx) occurs in three phases: inductive, angiogenic, and terminal. Biomechanical

forces within the liver sinusoid change after PHx including, shear stress, mechanical stretch, and changes in blood flow [151, 152], which stimulate endothelial proliferation promoting liver regeneration. A recent study demonstrated that hepatocyte proliferation could be induced by mechanical stretch induced YAP-dependent upregulation of heparin binding- epidermal growth factor (HB-EGF) in hepatic endothelial cells, with approximately a 2-fold increase after 3 hours of 10% stretch *in vitro*, and a 30-fold increase in vivo in LSECs at 48 hours following two-thirds PHx [151]. Moreover, LSECs (3% total liver volume) orchestrate regeneration via Notch-mediated, shear stress-induced pathways [97], releasing NO [153], downregulating S-adenosylmethionine (SAMe) [154], and modulating HGF and Wnt2 signals via VEGFR-2/ID1 [41]. During the angiogenic phase, angiopoietin 2 release stimulates TGFβ1 production, switching from hepatocyte to LSEC proliferation [155]. Hypoxia-induced VEGF and angiopoietins drive LSEC proliferation, peaking at 3–4 days post-PHx [156, 157]. Finally, LSECs secrete TGFβ1 to restore quiescence [155]. Thus, biomechanical stimuli are crucial for liver regeneration, and they directly modulate LSECs to produce regenerative signals [158].

In acute-on-chronic liver failure (ACLF), loss of fenestrae is marked due to sepsis or systemic inflammation that rapidly induce profound LSEC dysfunction, turning their phenotype to pro-inflammatory, pro-fibrotic, and vasoconstrictive. Sepsis-associated ACLF induces mice and human endothelial dysfunction by a reduced Angiopoietin-1/Angiopoietin-2 ratio. This stimulates the increases of LSEC-derived HGF, driving hepatocyte dedifferentiation and loss of function via activation of CCAAT/Enhancer-Binding Protein Beta (C/EBPβ). Targeting this axis may improve liver function and survival [159]. Single-cell transcriptomics in human ACLF further confirmed that vascular instability, impaired regeneration, and inflammation was supported by hepatic endothelial populations characterized by apoptotic and dysfunctional phenotype [160]. These endothelial alterations besides exacerbating hepatic injury, facilitate immune cell recruitment and systemic inflammatory responses, a known hallmark of ACLF [161]. Treatments aimed at reestablishing endothelial function like statins [35], could reduce portal pressure up to 40% in a model of ACLF, with marked reduction of neutrophils infiltration [162], confirming the crucial and hepato-protective role of LSECs also in acute hepatic failure.

4.5 LSECs in cholestatic liver diseases

LSECs are involved in the pathophysiology of cholestatic liver diseases, including primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) [163, 164]. Chronic inflammation and bile acid accumulation disrupt LSEC integrity and foster dedifferentiation; this may cause ischemic atrophy and hepatocytes injury [164, 165]. Activation of Liver X Receptor Alpha (LXRα), a bile acid receptor, prevents LSEC capillarization and HSC activation [166]. Thus, the imbalance of bile acids in cholestasis may be a driver of LSECs dysfunction [167]. The LSECs immunoregulatory role is crucial in PBC and PSC. Single cell analysis, observed that LSECs recruit monocytes after injury and foster a HMs-like phenotype, as demonstrated by doublet cells co-expressing macrophage markers (CD163, CD68, natural inhibitor of matrix metalloproteinases (TIMP1), Complement Component 1q (C1q)) and LSEC markers (Deoxyribonuclease 1 Like 3 (DNASE1L3), Endoglin (ENG), Secreted Protein Acidic and Rich in Cysteine (SPARC), C-Type Lectin Domain Family 1 Member B (CLEC1B)) [168]. Moreover, the colocalization occurred between HMs and central venous LSECs in normal donor and PSC human livers, confirmed by CD36 (endothelial) and C1QC (Kupffer) staining. Moreover, zonation of LSECs interaction via CCL21 with HSCs and $CD4^+$ T-cells was observed, mostly in periportal areas in both PSC and PBC [168].

4.6 LSECs in hepatocellular carcinoma

The progression of CLD to liver cancer involves precancerous lesions in which dedifferentiated LSECs influence tumor growth, metastasis, and the immune microenvironment [169]. Anti-angiogenic therapies like sorafenib, anti-Angiopoietin-2 peptibodies underscore the importance of LSEC-mediated angiogenesis as a therapeutic target [170, 171]. Recently the presence of two endothelial gene clusters was observed in hepatocellular carcinoma (HCC), one of which showed a high proliferative phenotype [172]. Peritumoral LSECs present angiogenic phenotype [173, 174], via secretion of VEGF, angiopoietin-2, and PDGF, driven by IL-6 signaling [174, 175]. In MASLD-associated HCC, Fatty Acid Binding Protein 4 (FABP4) is upregulated in peritumoral LSECs, promoting hepatocyte proliferation and angiogenesis via the mTOR pathway [176-178]. LSECs in HCC secrete CCL20 under hypoxia, recruiting regulatory T cells (Tregs) via CCR6, and expressing CXCL12, promoting metastasis through CXCR4 as recently reviewed [179]. Tumor-LSECs overexpress ManR, IL-1,

ICAM-1, and Cyclooxygenase-2 (COX-2), suppressing Interferon-gamma (IFN-γ) and inducing IL-10 [180].

5. LSECs and the immune response in liver diseases

LSECs play a crucial role in immune surveillance and tolerance by acting as gatekeepers between blood-borne mediators, liver parenchyma and circulating immune cells [181]. LSECs express pattern recognition receptors (PRRs) and scavenger receptors [182], enabling them to capture and process a wide variety of pathogen- and damage-associated molecular patterns (PAMPs and DAMPs) [182] filtered through their fenestrations, that will further be used as ligands for antigen presentation. They are also considered non-professional APCs to T cells in a manner that promotes immune tolerance rather than immunity in homeostatic conditions [183, 184]. Furthermore, LSECs produce and secrete inflammatory mediators including cytokines/chemokines to communicate with immune counterparts regulating their recruitment, activation, and tolerogenic state **(Figure 3)**.

5.1 Role in immune surveillance and tolerance

LSECs and Kupffer cells engage in dynamic crosstalk to maintain hepatic immune homeostasis. LSECs, through their expression of PD-L1, interact with PD-1 receptors on HMs to suppress excessive immune activation and maintain tolerance. Additionally, LSECs modulate HMs activity by producing anti-inflammatory cytokines such as IL-10, dampening pro-inflammatory responses [185, 186]. In the specific context of MASLD, it has been shown that the NO/cGMP/VASP signaling is an additional mechanism from LSEC to inhibit Kupffer cell activation [187]. Along the same lines, short-term exposure of LSEC to free fatty acids decreased the expression of pro-inflammatory chemokines (MCP1, CXCL10 and CXCL16), which are involved in monocyte and macrophage recruitment [188]. In homeostatic conditions, both LSEC and Kupffer cells cooperate in clearing pathogens, toxins, and apoptotic cells via PRR including Toll-like receptors [182]. Yao and colleagues quantified fluor-tagged LPS clearance and found that 75% of the LPS was cleared by LSEC while only around 25% was associated with Kupffer cells [189]. At the functional level, LPS tolerance in LSEC occurs through a downregulation of ICAM-1, reducing leukocyte adhesion and infiltration [190].This coordinated interaction ensures a fine balance between immune surveillance and tolerance, preventing excessive inflammation while enabling effective pathogen clearance in the liver sinusoids [191].

LSECs are specialized APCs uniquely adapted to maintain immune tolerance in the sinusoidal milieu. Unlike classical dendritic cells, LSECs present antigens in a tolerogenic manner, promoting T cell anergy, apoptosis, or the induction of Tregs [192]. The expression of scavenger receptors, major histocompatibility complex class I and II and costimulatory molecules like CD40, CD80, and CD86, is crucial for an efficient LSECs interaction and regulation with T cells [182]. Recent studies shed light on the cellular and molecular mechanisms mediated by LSEC to induce T cell anergy. While LSEC-T cell physical contact is necessary for inducing T-cell apoptosis (involving caspase 3, 8 and Bak), this effect is partially supported by TGFβ secretion from LSEC [193]. Additionally, B7-H1 overexpression by LSEC also results in tolerance in $CD8^+$ cells [33, 194]. LSEC have also been described to inhibit IFNγ, IL-2 and IL-17 production by T cells in an IL-10- and PD-1-dependent manner [195, 196]. Scavenger receptors like L-SIGN can recognize activated T cells and negatively regulate their proliferation, activation and production of T-cell cytokines in dendritic cells [197]. Tang and colleagues studied the role of LSECtin, an LSEC-specific scavenger receptor, and showed that mice with a genetic deletion of L-SIGN had increased T cell accumulation in the liver. In a model of acute liver injury induced by concavalin A, lack of LSECtin accelerated disease progression, exacerbating T cell response which was attenuated by exogenous administration of recombinant LSECtin [198, 199].

5.2 LSECs in liver inflammation

Adhesion molecules play a central role in the recruitment and activation of immune cells to the liver [181]. The expression of adhesion molecules (ICAM-1, VCAM-1) in LSECs facilitate the rolling, adhesion, crawling and transmigration of leukocytes from the bloodstream into the liver sinusoids and liver parenchyma [200]. This recruitment is also mediated by cytokine/chemokine signaling. CXCL9 and CXCL10 attract T cells, MCP-1 recruits CCR2+ monocyte-derived macrophages [1], CXCL1 production attracts neutrophils, and CXCL16 recruit CXCR6-NKT cells [134, 201]. Additional mediators have been detailed in Table 2.

NK cells are the early defense against infections and malignancies by exerting cytotoxicity and sustain inflammation via production of TNFα and IFNγ [202]. Papaioannou et al recently described the requirement of LSEC-derived CXCL10 after IFNγ exposure to accumulate activated NK cells in the inflamed liver upon LPS

treatment. They show that the interaction with LSECs leads to NK cell transcriptional reprogramming and priming for an enhanced IFNγ production [203].

In the context of inflammation during CLD or viral infection (e.g. COVID-19), capillarized LSEC produce an array of pro-inflammatory cytokines including TNFα, CCL3, CCL4, RANTES, CXCL1, CXCL2, MCAP1 and IFNγ to recruit immune cells to the injured liver as well as P-selectin, E-selectin and vWF, which are involved in platelet adhesion and microthrombi formation, NET release and contribute to the development of portal hypertension [134, 204]. In the context of adaptive immunity, capillarized LSECs gain enhanced capacity to capture Ag and induce T cell proliferation, enhancing CTL responses while preventing the generation of Tregs [62].

5.3 Immune evasion in liver cancer / metastasis

LSECs play a key role in immune tolerance, distinguishing between harmful pathogens and harmless antigens, a function that is compromised in cancer. Dysfunctional LSEC can promote immune evasion in HCC by altering immune cell recruitment and function. In contrast to LSECs from healthy livers, microvascular endothelial cells from human liver cancer tissue exhibit a different phenotype, including reduced expression of LSEC-specific markers (Stab1, Stab2, Lyve1 and CD32b), reduced ICAM-1 expression and increased production of tumor necrosis factor receptor (TNFR) p75, αvβ3, and αvβ5 integrins [169]. These changes impair immune cell adhesion and facilitate immune evasion. LSECs in the tumor microenvironment also promote TGFβ-dependent conversion of $Foxp3^-$ cells into $Foxp3^+$ Treg cells and NKT cells suppression via CXCL16-CXCR6 signaling, promoting an anti-tumor response [169, 205]. These responses shift LSEC antigen-presenting activity toward promoting T cell anergy or apoptosis, enhancing immune tolerance and supporting tumor progression [206]. Combining immune checkpoint inhibitors with anti-VEGF therapy may improve liver cancer outcomes by increasing T cell infiltration and reducing tumor immune evasion, benefiting from VEGF's role in promoting vascular and immune suppression [207].

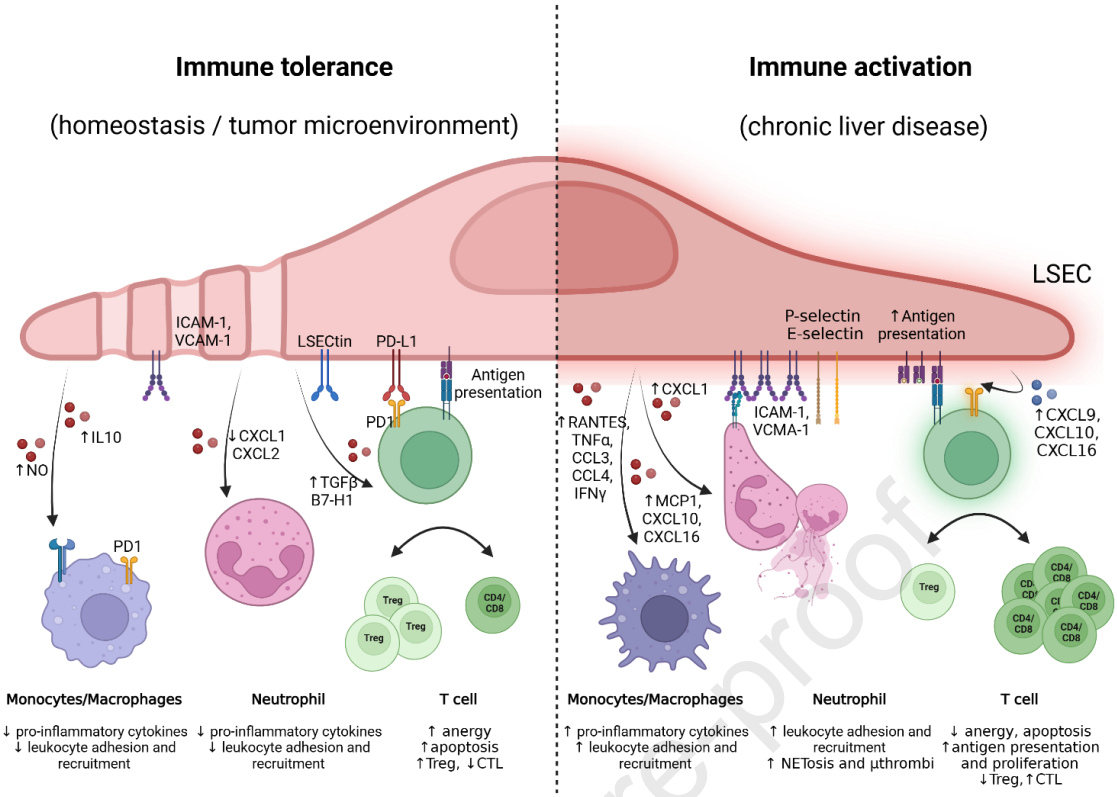

Figure 3. LSEC regulation of immune cells recruitment and activation in homeostasis and disease. Diagram depicting LSEC-induced immune tolerance molecular mechanisms occurring during homeostatic and tumor evasion conditions (left side) or LSEC-mediated immune cell activation (right side) during chronic liver disease. Immune cell regulation includes monocytes/macrophages, neutrophils and T cells. Abbreviations: B7-H1 (B7 homolog 1), CCx (chemokine (C-C motif), CDx (cluster of differentiation x), CTL (cytotoxic T lymphocyte), CXCLx (C-X-C motif chemokine ligand x), ICAM-1 (intercellular adhesion molecule 1), IFNγ (interferon gamma), IL10 (interleukin 10), LSEC (liver sinusoidal endothelial cell), LSECtin (liver and lymph node sinusoidal endothelial cell C-type lectin), MCP1 (monocyte chemoattractant protein 1), NETosis (neutrophil extracellular trap formation), NO (nitric oxide), PD-1 (programmed cell death protein 1), PD-L1 (programmed death-ligand 1), RANTES (regulated on activation, normal T cell expressed and secreted), TGFβ (transforming growth factor beta), TNFα (tumor necrosis factor alpha), Treg (regulatory T cell), VCAM-1 (vascular cell adhesion molecule 1).

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6. Therapeutic potential of targeting LSECs in liver diseases

As evidenced above, the role of LSECs in liver homeostasis is crucial. Maintaining a healthy phenotype of LSECs, namely porosity, anti-inflammatory and anti-fibrotic signaling, immunoregulatory role, crosstalk and their scavenging activity, is key for interrupting most of the pathological processes. As highlighted in recent literature **(Tables 1-3)**, LSEC-targeted therapeutic strategies may offer the opportunity for a shared approach that can be useful across any stage or type of liver disease.

Table 1. Therapeutic potential targets of LSEC in fibrosis & cirrhosis

Experimental model	Pathological condition	Molecular target	Mechanism (& therapeutic strategy)	Translational potential	Ref.
Wistar rat	CCl_4 induced cirrhosis	KLF2	KLF2 activation promotes LSEC re-differentiation *Drug: Simvastatin*	+++	[36]
Sprague Dawley rat	TAA or cBDL induced cirrhosis	PPAR	Pan-PPAR agonist reverses LSECs capillarization *Drug: Lanifibranor*	+++	[25]
C57BL/6 mice	CDAA diet-induced fibrosis	GATA4	GATA4 inhibits LSECs-derived profibrotic angiocrine signaling	++	[37]
C57BL/6 mice	MCD diet MASH	Notch	Inhibition of Notch restores LSECs-eNOS homeostasis *Drug: YC-1 (S7958)*	++	[208]
MISTRG6 mice	CCl_4 induced fibrosis	WNT2	LSECs-derived WNT2 regulates cholesterol to prevent fibrosis	++	[48]
Tie2-Cre mice	TAA induced fibrosis	RUNX3	RUNX3 protects LSECs by depleting LSECs-derived profibrotic LRG1	++	[209]
C57BL/6 mice	CCl_4 induced fibrosis	POFUT1	POFUT1 inhibits fibrosis by inhibiting LSECs-derived fibrinogen	++	[210]
Sprague Dawley rat	TAA induced cirrhosis	CIB1	Inhibition of CIB1 enhances LSECs re-differentiation	+	[72]
C57BL/6 mice	CDHFD induced MASH fibrosis	GSK3	Inhibition of GSK3 attenuates LSECs endotheliopathy *Drug: LY2090314*	++	[211]
F344/NSlc rat	CCl_4 induced cirrhosis	GPX	GPX enzyme enhances LSECs reducing reactive oxygen species *Drug: Tofogliflozin*	++	[212]

Note: All molecular targets and corresponding references are sequenced by publication date. Translational potential classified as follows: +++: Ongoing or completed clinical trials supported by prior preclinical animal model studies; ++: Preclinical animal model studies supported by prior in vitro studies; +: in vitro studies supported by omics analysis.

Abbreviation: KLF2, Krüppel-like factor 2; PPAR, peroxisome proliferator-activated receptors; GSK3, glycogen synthase kinase-3; GATA-4, GATA binding protein 4; Notch, Notch signaling pathway; WNT2; wingless-type MMTV integration site family, member 2; RUNX3, Runt-related transcription factor 3; LRG1, leucine-rich alpha-2-glycoprotein 1; POFUT1, protein O-fucosyltransferase 1; CIB1, calcium and integrin-binding protein 1; GPX, glutathione peroxidase family.

Table 2: Immune response modulation and drug delivery & target in LSEC

Study population	Pathological condition	Molecular target	Mechanism	Translational potential	Ref.
C57BL/6 mice	FFC diet induced MASH	VCAM1	Reduce VCAM-1 enhances the monocyte adhesion on LSECs	++	[106]
C57BL/6 mice	CCl_4 induced fibrosis	PFKP	Inhibition of PFKP reduces stiffness-induced CXCL1 in LSECs	++	[135]
C57BL/6 mice	FFC diet induced MASH	DUSP4	DUSP4 reduction in LSECs aggravates neutrophil activation	+	[213]
C57BL/6 mice	LPS induced acute liver inflammation	CXCL10	LSECs produces CXCL10 and supports NK cell recruitment	++	[203]
C57BL/6 mice	CCl_4 induced cirrhosis	LSECtin	LSECtin reduces proinflammatory TNF-α, IL-1β, IL-6, CCL2 in LSECs	++	[199]
Sprague Dawley rat	TAA induced cirrhosis	ERG	ERG reduces proinflammatory IL-1β in LSECs	+	[67]
C57BL/6 mice	Chronic hepatitis B virus infection	CD8 T cells	LSECs regulate CD8 T cells recognizing antigen	+++	[214]
CD-1 mice	MC38 cancer cell line induced carcinoma	Organosilica nanoparticles (OSCs)	OSCs co-localize with LSECs avoiding Kupffer-cell uptake	++	[215]

Note: All molecular targets and corresponding references are sequenced by publication date. Translational potential classified as follows: +++: Ongoing or completed clinical trials supported by prior preclinical animal model studies; ++: Preclinical animal model studies supported by prior in vitro studies; +: in vitro studies supported by omics analysis.

Abbreviation: VCAM1, vascular cell adhesion molecule 1; PFKP, phosphofructokinase 1 isoform P; DUSP4, dual-specificity phosphatase 4; LSECtin, tolerogenic C-type lectin; ERG, ETS-related gene.

Table 3: LSEC as therapeutic target in hepatocellular carcinoma

Study population	Pathological condition	Molecular target	Mechanism	Translational potential	Ref.
C57BL/6 mice	SV40 T-antigen induced carcinoma	Stabilin-2	Inhibition of stabilin-2 prevents LSECs-tumor adhesive interactions	++	[173]
C57BL/6 mice	Hepa1–6 cell induced carcinoma	IL-8	Inhibition of the IL8–CXCR1 axis reduces LSECs permeability	++	[216]
BALB/c mice	C26 cell induced carcinoma	COX-2	Inhibition of COX-2 reduces metastasis by reducing tumor/LSECs crosstalk	++	[217]
C57BL/6 mice	Hepa1-6 cell induced carcinoma	MMP9	Inhibition of MMP9 reduces LSECs gap formation and metastasis	++	[218]
C57BL/6 mice	Melanoma induced carcinoma	Lyve-1	Inhibition of Lyve-1 in LSECs reduces metastasis	++	[219]
Human HCC cell lines	LSECs *in vitro* co-culture with HepG2 cells	Galectin-1 (Gal1)	Gal1 promotes adhesion of HepG2 cells to LSECs	++	[220]

Note: All molecular targets and corresponding references are sequenced by publication date. Translational potential classified as follows: ++: Preclinical animal model studies supported by prior in vitro studies; +: in vitro studies supported by omics analysis. The higher evidence proof (+++) for HCC targeting specifically LSECs is lacking.

Abbreviation: COX-2, Cyclooxygenase-2; MMP9, matrix metallopeptidase 9; Lyve-1, lymphatic vessel endothelial hyaluronan receptor 1; Galectin-1, β-galactoside-binding lectins

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7. Challenges and future directions

7.1 Gaps in knowledge about LSECs in liver diseases

Although substantial progress has been made in elucidating the roles of LSECs in homeostasis and disease, significant gaps persist in our understanding of their pathobiology. A major limitation is the incomplete characterization of the molecular pathways governing LSEC dedifferentiation across distinct liver diseases. While capillarization, loss of fenestrations, enhanced leukocyte adhesion, and pro-fibrogenic signaling have been documented, the upstream regulators and context-dependent variations in these processes are not fully defined. In particular, the contributions of LSEC subpopulations and their spatial heterogeneity along the porto-central axis remain insufficiently explored, especially in the context of human liver diseases.

Furthermore, translating preclinical findings into effective clinical interventions is challenged by fundamental differences between human and rodent LSEC biology, including disparities in fenestrae architecture, scavenger receptor profiles, and regenerative capacity. Many experimental models fail to fully recapitulate the complex multicellular interactions, mechanical forces, and metabolic milieu of the diseased human liver. Moreover, the multifactorial nature of common liver diseases, such as MASH, viral hepatitis, and alcohol-related liver disease, further complicates the extrapolation of preclinical data to heterogeneous patient populations. Consequently, there is a pressing need for improved experimental systems and human-relevant models to close this translational gap [221, 222].

7.2 Innovative research tools

Advances in imaging, molecular profiling, and *in vitro* modeling are beginning to address these challenges. High-resolution intravital microscopy enables real-time visualization of LSEC dynamics, including fenestration alterations, immune cell interactions, and barrier function during liver injury [223]. Complementarily, scRNA-seq and spatial transcriptomics have uncovered previously unrecognized LSEC subtypes and spatially restricted molecular programs, enhancing our understanding of endothelial heterogeneity in the liver [17, 224, 225].

Emerging *in vitro* models offer additional platforms to study LSEC behavior under controlled conditions. The development of liver organoids incorporating LSECs, hepatocytes, and non-parenchymal cells provides three-dimensional structures that

recapitulate key aspects of hepatic microanatomy and cellular crosstalk [226, 227]. Liver-on-chip technologies, which integrate microfluidic flow with co-culture systems, allow precise control over shear stress, nutrient gradients, mechanobiological cues, and inflammatory stimuli, enabling the study of mechanotransduction and endothelial responses in a physiologically relevant context [75, 228]. These platforms not only facilitate mechanistic investigations but also serve as preclinical testing beds for therapeutic candidates aimed at restoring or preserving LSEC function, especially when combined when primary human cell cultures.

7.3 Advances in LSECs-nanotargeting

Targeting LSECs without off-target effects in liver disease is a crucial and challenging goal. Key difficulties are represented by the capillarization, the lack of unique biomarker to target, altered receptor expression and phenotype reducing their ability to absorb drugs and carriers, a rapid clearance and competition with KCs. Several carriers have been designed in the last decade with promising results to overcome these challenges. Self-assembled lipid nanoparticles (LNP) represent one prominent approach, as demonstrated by α-melittin-NPs [223]. Quantum dots-based nanoparticles (silver-sulfide dots) application could successfully target LSECs in cirrhosis model [145]. Hyaluronic acid-modified polymeric nanoparticles (HA-NPs) have emerged as another successful strategy, leveraging the natural affinity of hyaluronic acid for CD44 receptors [229]. Poly(alkyl-cyanoacrylate) (PACA) nanoparticles offer a distinct targeting mechanism through stabilin-2 receptor-mediated uptake [230], while polymeric micelles (PMs) represent a more sophisticated targeting approach [231], incorporating specific peptide ligands targeting multiple LSEC receptors including CD32b, CD36, and ITGB3 [232]. The most recent advancement involves spherical nucleic acid (SNA) nanoparticles, specifically targeting the scavenger receptor A (Scara) that is highly overexpressed in fibrotic LSECs via clathrin-dependent endocytosis [233]. Finally, Topas particle conjugates (TPCs) represent a clinical-stage platform utilizing 25-30 nm nanoparticles with proprietary linker technology for diverse peptide conjugation, achieving preferential LSEC targeting for antigen-specific immunotherapy [234]. Together, these advances highlight the growing potential of nanotechnology to achieve precise and selective modulation of LSECs in liver disease.

7.4 Potential for precision medicine approaches

The growing body of knowledge on LSEC biology offers promising avenues for the development of precision medicine strategies in liver diseases [235]. As described above, LSECs are uniquely positioned as early sensors, mediators and responders of liver injury, and their dysfunction precedes many of the pathological features of progressive liver disease. Accordingly, LSEC-derived biomarkers, including soluble adhesion molecules, angiocrine factors, and extracellular vesicles, are being investigated as minimally invasive indicators of early endothelial injury and fibrotic progression [75, 104].

Moreover, the prospect of therapeutically targeting LSECs in a patient-specific manner is gaining traction. Detailed molecular profiling of LSEC dysfunction across different etiologies could enable the stratification of patients based on endothelial phenotypes, guiding tailored interventions. For example, therapies aimed at preserving fenestrations, modulating endothelial inflammation, or enhancing regenerative angiocrine signaling may be selectively deployed according to the dominant pathological processes in individual patients [236]. Such precision approaches could mitigate the limitations of current therapies, which often fail to address the endothelial contribution to liver disease pathogenesis.

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8. Conclusions

LSECs are central regulators of hepatic homeostasis and disease. Their unique structural and functional characteristics, including high porosity, scavenger, vasoactive, angiocrine and thrombotic activities, and immunomodulatory roles, are increasingly recognized as key determinants of liver health. Dysregulation of LSECs—marked by capillarization, inflammatory activation, and loss of barrier function—contributes significantly to the progression of chronic liver diseases such as fibrosis, cirrhosis, and hepatocellular carcinoma. However, major gaps remain in our understanding of the molecular mechanisms underlying LSEC dysfunction and translating experimental insights into clinical therapies remains a challenge. Recent advances in single-cell technologies and new models/tools offer opportunities to study LSECs in more physiologically relevant contexts. The identification of LSEC-derived biomarkers and the development of targeted therapies may enable earlier diagnosis and personalized interventions. Further research into LSEC biology holds the potential to shift current approaches to liver disease management, positioning LSECs as critical therapeutic targets in the coming years.

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Note from the authors: We would like to dedicate this review to the memory of Professor Jaume Bosch, whose pioneering contributions profoundly shaped our understanding of liver pathophysiology and vascular diseases. Beyond his remarkable scientific legacy, his generosity, mentorship, and vision continue to inspire the hepatology community. His work will remain a guiding light for generations to come, and his influence will be felt in every step forward in the field.

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References

[1] Gracia-Sancho J, Marrone G, Fernandez-Iglesias A. Hepatic microcirculation and mechanisms of portal hypertension. Nat Rev Gastroenterol Hepatol 2019;16:221-234.

[2] **Gracia-Sancho J, Caparros E, Fernandez-Iglesias A,** et al. Role of liver sinusoidal endothelial cells in liver diseases. Nat Rev Gastroenterol Hepatol 2021;18:411-431.

[3] Deleve LD, Wang X, Guo Y. Sinusoidal endothelial cells prevent rat stellate cell activation and promote reversion to quiescence. Hepatology 2008;48:920-930.

[4] Rockey DC, Weisiger RA. Endothelin induced contractility of stellate cells from normal and cirrhotic rat liver: implications for regulation of portal pressure and resistance. Hepatology 1996;24:233-240.

[5] Do H, Healey JF, Waller EK, et al. Expression of factor VIII by murine liver sinusoidal endothelial cells. J Biol Chem 1999;274:19587-19592.

[6] **Kume M, Hayashi T**, Yuasa H, et al. Bacterial lipopolysaccharide decreases thrombomodulin expression in the sinusoidal endothelial cells of rats -- a possible mechanism of intrasinusoidal microthrombus formation and liver dysfunction. J Hepatol 2003;38:9-17.

[7] Wisse E. An electron microscopic study of the fenestrated endothelial lining of rat liver sinusoids. J Ultrastruct Res 1970;31:125-150.

[8] Wisse E, De Zanger RB, et al. Scanning electron microscope observations on the structure of portal veins, sinusoids and central veins in rat liver. Scan Electron Microsc 1983:1441-1452.

[9] **Wisse E, Jacobs F,** Topal B, et al. The size of endothelial fenestrae in human liver sinusoids: implications for hepatocyte-directed gene transfer. Gene Ther 2008;15:1193-1199.

[10] Braet F, Spector I, De Zanger R, et al. A novel structure involved in the formation of liver endothelial cell fenestrae revealed by using the actin inhibitor misakinolide. Proc Natl Acad Sci U S A 1998;95:13635-13640.

[11] Zapotoczny B, Braet F, Kus E, et al. Actin-spectrin scaffold supports open fenestrae in liver sinusoidal endothelial cells. Traffic 2019;20:932-942.

[12] Czyzynska-Cichon I, Giergiel M, Kwiatkowski G, et al. Protein disulfide isomerase A1 regulates fenestration dynamics in primary mouse liver sinusoidal endothelial cells (LSECs). Redox Biol 2024;72:103162.

[13] Zhang H, Pu W, Tian X, et al. Genetic lineage tracing identifies endocardial origin of liver vasculature. Nat Genet 2016;48:537-543.

[14] **MacParland SA, Liu JC, Ma XZ,** et al. Single cell RNA sequencing of human liver reveals distinct intrahepatic macrophage populations. Nat Commun 2018;9:4383.

[15] Aizarani N, Saviano A, Sagar, et al. A human liver cell atlas reveals heterogeneity and epithelial progenitors. Nature 2019;572:199-204.

[16] Strauss O, Phillips A, Ruggiero K, et al. Immunofluorescence identifies distinct subsets of endothelial cells in the human liver. Sci Rep 2017;7:44356.

[17] Su T, Yang Y, Lai S, et al. Single-Cell Transcriptomics Reveals Zone-Specific Alterations of Liver Sinusoidal Endothelial Cells in Cirrhosis. Cell Mol Gastroenterol Hepatol 2021;11:1139-1161.

[18] **Tian SP, Ge JY,** Song YM, et al. A novel efficient strategy to generate liver sinusoidal endothelial cells from human pluripotent stem cells. Sci Rep 2024;14:13831.

[19] **Ning M, Lu D,** Liang D, et al. Single-cell RNA sequencing advances in revealing the development and progression of MASH: the identifications and interactions of non-parenchymal cells. Front Mol Biosci 2025;12:1513993.

[20] de Haan W, Oie C, Benkheil M, et al. Unraveling the transcriptional determinants of liver sinusoidal endothelial cell specialization. Am J Physiol Gastrointest Liver Physiol 2020;318:G803-G815.

[21] Ramachandran P, Dobie R, Wilson-Kanamori JR, et al. Resolving the fibrotic niche of human liver cirrhosis at single-cell level. Nature 2019;575:512-518.

[22] **Li ZW, Ruan B, Yang PJ**, et al. Oit3, a promising hallmark gene for targeting liver sinusoidal endothelial cells. Signal Transduct Target Ther 2023;8:344.

[23] McConnell MJ, Kostallari E, Ibrahim SH, et al. The evolving role of liver sinusoidal endothelial cells in liver health and disease. Hepatology 2023;78:649-669.
[24] Maeso-Diaz R, Ortega-Ribera M, Lafoz E, et al. Aging Influences Hepatic Microvascular Biology and Liver Fibrosis in Advanced Chronic Liver Disease. Aging Dis 2019;10:684-698.
[25] **Boyer-Diaz Z, Aristu-Zabalza P**, Andres-Rozas M, et al. Pan-PPAR agonist lanifibranor improves portal hypertension and hepatic fibrosis in experimental advanced chronic liver disease. J Hepatol 2021;74:1188-1199.
[26] Zapotoczny B, Braet F, Wisse E, et al. Biophysical nanocharacterization of liver sinusoidal endothelial cells through atomic force microscopy. Biophys Rev 2020;12:625-636.
[27] **Monkemoller V, Oie C**, Hubner W, et al. Multimodal super-resolution optical microscopy visualizes the close connection between membrane and the cytoskeleton in liver sinusoidal endothelial cell fenestrations. Sci Rep 2015;5:16279.
[28] Thomson AW, Knolle PA. Antigen-presenting cell function in the tolerogenic liver environment. Nat Rev Immunol 2010;10:753-766.
[29] Magnusson S, Berg T. Extremely rapid endocytosis mediated by the mannose receptor of sinusoidal endothelial rat liver cells. Biochem J 1989;257:651-656.
[30] Gomez-Salinero JM, Redmond D, Rafii S. Microenvironmental determinants of endothelial cell heterogeneity. Nat Rev Mol Cell Biol 2025;26:476-495.
[31] Knolle PA, Schmitt E, Jin S, et al. Induction of cytokine production in naive CD4(+) T cells by antigen-presenting murine liver sinusoidal endothelial cells but failure to induce differentiation toward Th1 cells. Gastroenterology 1999;116:1428-1440.
[32] **Wittlich M, Dudek M**, Bottcher JP, et al. Liver sinusoidal endothelial cell cross-priming is supported by CD4 T cell-derived IL-2. J Hepatol 2017;66:978-986.
[33] **Limmer A, Ohl J**, Kurts C, et al. Efficient presentation of exogenous antigen by liver endothelial cells to CD8+ T cells results in antigen-specific T-cell tolerance. Nat Med 2000;6:1348-1354.
[34] Marrone G, Shah VH, Gracia-Sancho J. Sinusoidal communication in liver fibrosis and regeneration. J Hepatol 2016;65:608-617.
[35] **Marrone G, Russo L**, Rosado E, et al. The transcription factor KLF2 mediates hepatic endothelial protection and paracrine endothelial-stellate cell deactivation induced by statins. J Hepatol 2013;58:98-103.
[36] **Marrone G, Maeso-Diaz R**, Garcia-Cardena G, et al. KLF2 exerts antifibrotic and vasoprotective effects in cirrhotic rat livers: behind the molecular mechanisms of statins. Gut 2015;64:1434-1443.
[37] **Winkler M, Staniczek T, Kurschner SW,** et al. Endothelial GATA4 controls liver fibrosis and regeneration by preventing a pathogenic switch in angiocrine signaling. J Hepatol 2021;74:380-393.
[38] **Schmid CD, Olsavszky V, Reinhart M**, et al. ALK1 controls hepatic vessel formation, angiodiversity, and angiocrine functions in hereditary hemorrhagic telangiectasia of the liver. Hepatology 2023;77:1211-1227.
[39] Preziosi M, Okabe H, Poddar M, et al. Endothelial Wnts regulate beta-catenin signaling in murine liver zonation and regeneration: A sequel to the Wnt-Wnt situation. Hepatol Commun 2018;2:845-860.
[40] LeCouter J, Moritz DR, Li B, et al. Angiogenesis-independent endothelial protection of liver: role of VEGFR-1. Science 2003;299:890-893.
[41] Ding BS, Nolan DJ, Butler JM, et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. Nature 2010;468:310-315.
[42] Gracia-Sancho J, Guixe-Muntet S. The many-faced role of autophagy in liver diseases. J Hepatol 2018;68:593-594.
[43] **Oteiza A, Li R**, McCuskey RS, et al. Effects of oxidized low-density lipoproteins on the hepatic microvasculature. Am J Physiol Gastrointest Liver Physiol 2011;301:G684-693.

[44] Steinberg D. Low density lipoprotein oxidation and its pathobiological significance. J Biol Chem 1997;272:20963-20966.
[45] **Li R, Oteiza A**, Sorensen KK, et al. Role of liver sinusoidal endothelial cells and stabilins in elimination of oxidized low-density lipoproteins. Am J Physiol Gastrointest Liver Physiol 2011;300:G71-81.
[46] **Zhang Q, Liu J**, Liu J, et al. oxLDL induces injury and defenestration of human liver sinusoidal endothelial cells via LOX1. J Mol Endocrinol 2014;53:281-293.
[47] **Zhang Q, Lin W**, Tian L, et al. Oxidized low-density lipoprotein activates extracellular signal-regulated kinase signaling to downregulate sortilin expression in liver sinusoidal endothelial cells. J Gastroenterol Hepatol 2021;36:2610-2618.
[48] Kaffe E, Roulis M, Zhao J, et al. Humanized mouse liver reveals endothelial control of essential hepatic metabolic functions. Cell 2023;186:3793-3809 e3726.
[49] **Czyzynska-Cichon I, Kotlinowski J**, Blacharczyk O, et al. Early and late phases of liver sinusoidal endothelial cell (LSEC) defenestration in mouse model of systemic inflammation. Cell Mol Biol Lett 2024;29:139.
[50] Hahn E, Wick G, Pencev D, et al. Distribution of basement membrane proteins in normal and fibrotic human liver: collagen type IV, laminin, and fibronectin. Gut 1980;21:63-71.
[51] DeLeve LD, Wang X, Hu L, et al. Rat liver sinusoidal endothelial cell phenotype is maintained by paracrine and autocrine regulation. Am J Physiol Gastrointest Liver Physiol 2004;287:G757-763.
[52] Xie G, Wang X, Wang L, et al. Role of differentiation of liver sinusoidal endothelial cells in progression and regression of hepatic fibrosis in rats. Gastroenterology 2012;142:918-927 e916.
[53] Desroches-Castan A, Tillet E, Ricard N, et al. Differential Consequences of Bmp9 Deletion on Sinusoidal Endothelial Cell Differentiation and Liver Fibrosis in 129/Ola and C57BL/6 Mice. Cells 2019;8.
[54] **Luo X, Wang D, Zhu X**, et al. Author Correction: Autophagic degradation of caveolin-1 promotes liver sinusoidal endothelial cells defenestration. Cell Death Dis 2019;10:482.
[55] Felli E, Felli E, Muttillo EM, et al. Liver ischemia-reperfusion injury: From trigger loading to shot firing. Liver Transpl 2023;29:1226-1233.
[56] **Yazdani HO, Chen HW**, Tohme S, et al. IL-33 exacerbates liver sterile inflammation by amplifying neutrophil extracellular trap formation. J Hepatol 2017.
[57] Xie G, Choi SS, Syn WK, et al. Hedgehog signalling regulates liver sinusoidal endothelial cell capillarisation. Gut 2013;62:299-309.
[58] **Duan JL, Ruan B, Yan XC**, et al. Endothelial Notch activation reshapes the angiocrine of sinusoidal endothelia to aggravate liver fibrosis and blunt regeneration in mice. Hepatology 2018;68:677-690.
[59] **Eberhard D, Balkenhol S**, Koster A, et al. Semaphorin-3A regulates liver sinusoidal endothelial cell porosity and promotes hepatic steatosis. Nat Cardiovasc Res 2024;3:734-753.
[60] DeLeve LD. Liver sinusoidal endothelial cells and liver regeneration. J Clin Invest 2013;123:1861-1866.
[61] Baiocchini A, Del Nonno F, Taibi C, et al. Publisher Correction: Liver sinusoidal endothelial cells (LSECs) modifications in patients with chronic hepatitis C. Sci Rep 2020;10:1420.
[62] Connolly MK, Bedrosian AS, Malhotra A, et al. In hepatic fibrosis, liver sinusoidal endothelial cells acquire enhanced immunogenicity. J Immunol 2010;185:2200-2208.
[63] Miyao M, Kotani H, Ishida T, et al. Pivotal role of liver sinusoidal endothelial cells in NAFLD/NASH progression. Lab Invest 2015;95:1130-1144.
[64] **Felli E, Selicean S, Guixe-Muntet S**, et al. Mechanobiology of portal hypertension. JHEP Rep 2023;5:100869.
[65] **Guixe-Muntet S, Ortega-Ribera M**, Wang C, et al. Nuclear deformation mediates liver cell mechanosensing in cirrhosis. JHEP Rep 2020;2:100145.

[66] Dufton NP, Peghaire CR, Osuna-Almagro L, et al. Author Correction: Dynamic regulation of canonical TGFbeta signalling by endothelial transcription factor ERG protects from liver fibrogenesis. Nat Commun 2020;11:1301.
[67] **Selicean SE, Felli E**, Wang C, et al. Stiffness-induced modulation of ERG transcription factor in chronic liver disease. NPJ Gut Liver 2024;1:7.
[68] Dufton NP, Peghaire CR, Osuna-Almagro L, et al. Dynamic regulation of canonical TGFbeta signalling by endothelial transcription factor ERG protects from liver fibrogenesis. Nat Commun 2017;8:895.
[69] Wells RG. The role of matrix stiffness in regulating cell behavior. Hepatology 2008;47:1394-1400.
[70] Maniotis AJ, Chen CS, Ingber DE. Demonstration of mechanical connections between integrins, cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. Proc Natl Acad Sci U S A 1997;94:849-854.
[71] **Fu M, Hu Y, Lan T**, et al. Correction: The Hippo signalling pathway and its implications in human health and diseases. Signal Transduct Target Ther 2024;9:5.
[72] **Wang C, Felli E**, Selicean S, et al. Role of calcium integrin-binding protein 1 in the mechanobiology of the liver endothelium. J Cell Physiol 2024;239:e31198.
[73] Wedgwood S, Mitchell CJ, Fineman JR, et al. Developmental differences in the shear stress-induced expression of endothelial NO synthase: changing role of AP-1. Am J Physiol Lung Cell Mol Physiol 2003;284:L650-662.
[74] Morawietz H, Wagner AH, Hecker M, et al. Endothelin receptor B-mediated induction of c-jun and AP-1 in response to shear stress in human endothelial cells. Can J Physiol Pharmacol 2008;86:499-504.
[75] **Ortega-Ribera M, Gibert-Ramos A**, Abad-Jorda L, et al. Increased sinusoidal pressure impairs liver endothelial mechanosensing, uncovering novel biomarkers of portal hypertension. JHEP Rep 2023;5:100722.
[76] Walker CJ, Crocini C, Ramirez D, et al. Nuclear mechanosensing drives chromatin remodelling in persistently activated fibroblasts. Nat Biomed Eng 2021;5:1485-1499.
[77] Caldwell S, Ikura Y, Dias D, et al. Hepatocellular ballooning in NASH. J Hepatol 2010;53:719-723.
[78] **Wisse E, Braet F**, Shami GJ, et al. Fat causes necrosis and inflammation in parenchymal cells in human steatotic liver. Histochem Cell Biol 2022;157:27-38.
[79] Hammoutene A, Rautou PE. Role of liver sinusoidal endothelial cells in non-alcoholic fatty liver disease. J Hepatol 2019;70:1278-1291.
[80] Blouin A, Bolender RP, Weibel ER. Distribution of organelles and membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. A stereological study. J Cell Biol 1977;72:441-455.
[81] Martinez I, Nedredal GI, Oie CI, et al. The influence of oxygen tension on the structure and function of isolated liver sinusoidal endothelial cells. Comp Hepatol 2008;7:4.
[82] Gracia-Sancho J, Lavina B, Rodriguez-Vilarrupla A, et al. Evidence against a role for NADPH oxidase modulating hepatic vascular tone in cirrhosis. Gastroenterology 2007;133:959-966.
[83] Gracia-Sancho J, Lavina B, Rodriguez-Vilarrupla A, et al. Increased oxidative stress in cirrhotic rat livers: A potential mechanism contributing to reduced nitric oxide bioavailability. Hepatology 2008;47:1248-1256.
[84] Mooli RGR, Mukhi D, Ramakrishnan SK. Oxidative Stress and Redox Signaling in the Pathophysiology of Liver Diseases. Compr Physiol 2022;12:3167-3192.
[85] Hamer I, Wattiaux R, Wattiaux-De Coninck S. Deleterious effects of xanthine oxidase on rat liver endothelial cells after ischemia/reperfusion. Biochim Biophys Acta 1995;1269:145-152.
[86] Corpechot C, Barbu V, Wendum D, et al. Hypoxia-induced VEGF and collagen I expressions are associated with angiogenesis and fibrogenesis in experimental cirrhosis. Hepatology 2002;35:1010-1021.

[87] Rosmorduc O, Wendum D, Corpechot C, et al. Hepatocellular hypoxia-induced vascular endothelial growth factor expression and angiogenesis in experimental biliary cirrhosis. Am J Pathol 1999;155:1065-1073.
[88] Maeso-Diaz R, Gracia-Sancho J. Aging and Chronic Liver Disease. Semin Liver Dis 2020;40:373-384.
[89] Baiocchi L, Glaser S, Francis H, et al. Impact of Aging on Liver Cells and Liver Disease: Focus on the Biliary and Vascular Compartments. Hepatol Commun 2021;5:1125-1137.
[90] **Sanfeliu-Redondo D, Gibert-Ramos A**, Gracia-Sancho J. Cell senescence in liver diseases: pathological mechanism and theranostic opportunity. Nat Rev Gastroenterol Hepatol 2024;21:477-492.
[91] Felli E, Gracia-Sancho J. Liver aging. In: Martins P, Baptista PM, Orlando G, editors. Regenerative Hepatology and Liver Transplantation: Academic Press; 2025. p. 391-408.
[92] Maeso-Diaz R, Ortega-Ribera M, Fernandez-Iglesias A, et al. Effects of aging on liver microcirculatory function and sinusoidal phenotype. Aging Cell 2018;17:e12829.
[93] Le Couteur DG, Cogger VC, Markus AM, et al. Pseudocapillarization and associated energy limitation in the aged rat liver. Hepatology 2001;33:537-543.
[94] Warren A, Bertolino P, Cogger VC, et al. Hepatic pseudocapillarization in aged mice. Exp Gerontol 2005;40:807-812.
[95] Gracia-Sancho J, Garcia-Caldero H, Hide D, et al. Simvastatin maintains function and viability of steatotic rat livers procured for transplantation. J Hepatol 2013;58:1140-1146.
[96] **Luo X, Bai Y, He S**, et al. Sirtuin 1 ameliorates defenestration in hepatic sinusoidal endothelial cells during liver fibrosis via inhibiting stress-induced premature senescence. Cell Prolif 2021;54:e12991.
[97] Duan JL, Ruan B, Song P, et al. Shear stress-induced cellular senescence blunts liver regeneration through Notch-sirtuin 1-P21/P16 axis. Hepatology 2022;75:584-599.
[98] **Bhandari S, Larsen AK**, McCourt P, et al. The Scavenger Function of Liver Sinusoidal Endothelial Cells in Health and Disease. Front Physiol 2021;12:757469.
[99] Simon-Santamaria J, Malovic I, Warren A, et al. Age-related changes in scavenger receptor-mediated endocytosis in rat liver sinusoidal endothelial cells. J Gerontol A Biol Sci Med Sci 2010;65:951-960.
[100] **Wan Y, Li X, Slevin E**, et al. Endothelial dysfunction in pathological processes of chronic liver disease during aging. FASEB J 2022;36:e22125.
[101] Grosse L, Bulavin DV. LSEC model of aging. Aging (Albany NY) 2020;12:11152-11160.
[102] **Vats R, Li Z**, Ju EM, et al. Intravital imaging reveals inflammation as a dominant pathophysiology of age-related hepatovascular changes. Am J Physiol Cell Physiol 2022;322:C508-C520.
[103] Younossi ZM, Kalligeros M, Henry L. Epidemiology of Metabolic Dysfunction-Associated Steatotic Liver Disease. Clin Mol Hepatol 2024.
[104] **Gao J, Lan T, Kostallari E**, et al. Angiocrine signaling in sinusoidal homeostasis and liver diseases. J Hepatol 2024;81:543-561.
[105] Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018;24:908-922.
[106] Furuta K, Guo Q, Pavelko KD, et al. Lipid-induced endothelial vascular cell adhesion molecule 1 promotes nonalcoholic steatohepatitis pathogenesis. J Clin Invest 2021;131.
[107] Montagnani M, Chen H, Barr VA, et al. Insulin-stimulated activation of eNOS is independent of Ca2+ but requires phosphorylation by Akt at Ser(1179). J Biol Chem 2001;276:30392-30398.
[108] Pasarin M, La Mura V, Gracia-Sancho J, et al. Sinusoidal endothelial dysfunction precedes inflammation and fibrosis in a model of NAFLD. PLoS One 2012;7:e32785.
[109] Cogger VC, Mohamad M, Solon-Biet SM, et al. Dietary macronutrients and the aging liver sinusoidal endothelial cell. Am J Physiol Heart Circ Physiol 2016;310:H1064-1070.

[110] **Zhou D, Zhang J, Xiao C**, et al. Trimethylamine-N-oxide (TMAO) mediates the crosstalk between the gut microbiota and hepatic vascular niche to alleviate liver fibrosis in nonalcoholic steatohepatitis. Front Immunol 2022;13:964477.
[111] Cogger VC, Hilmer SN, Sullivan D, et al. Hyperlipidemia and surfactants: the liver sieve is a link. Atherosclerosis 2006;189:273-281.
[112] Herrnberger L, Hennig R, Kremer W, et al. Formation of fenestrae in murine liver sinusoids depends on plasmalemma vesicle-associated protein and is required for lipoprotein passage. PLoS One 2014;9:e115005.
[113] Chalasani N, Wilson L, Kleiner DE, et al. Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. J Hepatol 2008;48:829-834.
[114] **Lee YA, Wallace MC**, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut 2015;64:830-841.
[115] Fernandez-Alvarez A, Alvarez MS, Gonzalez R, et al. Human SREBP1c expression in liver is directly regulated by peroxisome proliferator-activated receptor alpha (PPARalpha). J Biol Chem 2011;286:21466-21477.
[116] Gao B, Ahmad MF, Nagy LE, et al. Inflammatory pathways in alcoholic steatohepatitis. J Hepatol 2019;70:249-259.
[117] Shepard BD, Joseph RA, Kannarkat GT, et al. Alcohol-induced alterations in hepatic microtubule dynamics can be explained by impaired histone deacetylase 6 function. Hepatology 2008;48:1671-1679.
[118] Yang Y, Sangwung P, Kondo R, et al. Alcohol-induced Hsp90 acetylation is a novel driver of liver sinusoidal endothelial dysfunction and alcohol-related liver disease. J Hepatol 2021;75:377-386.
[119] Yeligar S, Tsukamoto H, Kalra VK. Ethanol-induced expression of ET-1 and ET-BR in liver sinusoidal endothelial cells and human endothelial cells involves hypoxia-inducible factor-1alpha and microrNA-199. J Immunol 2009;183:5232-5243.
[120] Schoepflin ZR, Shapiro IM, Risbud MV. Class I and IIa HDACs Mediate HIF-1alpha Stability Through PHD2-Dependent Mechanism, While HDAC6, a Class IIb Member, Promotes HIF-1alpha Transcriptional Activity in Nucleus Pulposus Cells of the Intervertebral Disc. J Bone Miner Res 2016;31:1287-1299.
[121] Sandahl TD, Gronbaek H, Moller HJ, et al. Hepatic macrophage activation and the LPS pathway in patients with alcoholic hepatitis: a prospective cohort study. Am J Gastroenterol 2014;109:1749-1756.
[122] Xia Y, Yang J, Sanyal AJ, et al. Persistent Hyperactivation of Endothelial Cells in Patients with Alcoholic Hepatitis. Alcohol Clin Exp Res 2020;44:1075-1087.
[123] Bautista AP. Neutrophilic infiltration in alcoholic hepatitis. Alcohol 2002;27:17-21.
[124] **Liu M, Cao S**, He L, et al. Super enhancer regulation of cytokine-induced chemokine production in alcoholic hepatitis. Nat Commun 2021;12:4560.
[125] Vijayalekshmi B, Sharma A, Prabhu SB, et al. Reticuloendothelial activation correlates with disease severity and predicts mortality in severe alcoholic hepatitis. Eur J Gastroenterol Hepatol 2021;33:e329-e334.
[126] McConnell MJ, Kondo R, Kawaguchi N, et al. Covid-19 and Liver Injury: Role of Inflammatory Endotheliopathy, Platelet Dysfunction, and Thrombosis. Hepatol Commun 2022;6:255-269.
[127] Miller AM, Wang H, Park O, et al. Anti-inflammatory and anti-apoptotic roles of endothelial cell STAT3 in alcoholic liver injury. Alcohol Clin Exp Res 2010;34:719-725.
[128] Gracia-Sancho J, Lavina B, Rodriguez-Vilarrupla A, et al. Enhanced vasoconstrictor prostanoid production by sinusoidal endothelial cells increases portal perfusion pressure in cirrhotic rat livers. J Hepatol 2007;47:220-227.
[129] Yu Q, Shao R, Qian HS, et al. Gene transfer of the neuronal NO synthase isoform to cirrhotic rat liver ameliorates portal hypertension. J Clin Invest 2000;105:741-748.

[130] Abraldes JG, Albillos A, Banares R, et al. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomized controlled trial. Gastroenterology 2009;136:1651-1658.
[131] Guixe-Muntet S, de Mesquita FC, Vila S, et al. Cross-talk between autophagy and KLF2 determines endothelial cell phenotype and microvascular function in acute liver injury. J Hepatol 2017;66:86-94.
[132] Liu S, Premont RT, Rockey DC. Endothelial nitric-oxide synthase (eNOS) is activated through G-protein-coupled receptor kinase-interacting protein 1 (GIT1) tyrosine phosphorylation and Src protein. J Biol Chem 2014;289:18163-18174.
[133] **Ding BS, Cao Z**, Lis R, et al. Divergent angiocrine signals from vascular niche balance liver regeneration and fibrosis. Nature 2014;505:97-102.
[134] Hilscher MB, Sehrawat T, Arab JP, et al. Mechanical Stretch Increases Expression of CXCL1 in Liver Sinusoidal Endothelial Cells to Recruit Neutrophils, Generate Sinusoidal Microthombi, and Promote Portal Hypertension. Gastroenterology 2019;157:193-209 e199.
[135] **Greuter T, Yaqoob U**, Gan C, et al. Mechanotransduction-induced glycolysis epigenetically regulates a CXCL1-dominant angiocrine signaling program in liver sinusoidal endothelial cells in vitro and in vivo. J Hepatol 2022;77:723-734.
[136] Gao J, Wei B, Liu M, et al. Endothelial p300 Promotes Portal Hypertension and Hepatic Fibrosis Through C-C Motif Chemokine Ligand 2-Mediated Angiocrine Signaling. Hepatology 2021;73:2468-2483.
[137] **Biecker E, Trebicka J**, Kang A, et al. Treatment of bile duct-ligated rats with the nitric oxide synthase transcription enhancer AVE 9488 ameliorates portal hypertension. Liver Int 2008;28:331-338.
[138] Matei V, Rodriguez-Vilarrupla A, Deulofeu R, et al. Three-day tetrahydrobiopterin therapy increases in vivo hepatic NOS activity and reduces portal pressure in CCl4 cirrhotic rats. J Hepatol 2008;49:192-197.
[139] Matei V, Rodriguez-Vilarrupla A, Deulofeu R, et al. The eNOS cofactor tetrahydrobiopterin improves endothelial dysfunction in livers of rats with CCl4 cirrhosis. Hepatology 2006;44:44-52.
[140] Schwabl P, Hambruch E, Seeland BA, et al. The FXR agonist PX20606 ameliorates portal hypertension by targeting vascular remodelling and sinusoidal dysfunction. J Hepatol 2017;66:724-733.
[141] Schwabl P, Brusilovskaya K, Supper P, et al. The soluble guanylate cyclase stimulator riociguat reduces fibrogenesis and portal pressure in cirrhotic rats. Sci Rep 2018;8:9372.
[142] **Kitao A, Sato Y**, Sawada-Kitamura S, et al. Endothelial to mesenchymal transition via transforming growth factor-beta1/Smad activation is associated with portal venous stenosis in idiopathic portal hypertension. Am J Pathol 2009;175:616-626.
[143] Ribera J, Pauta M, Melgar-Lesmes P, et al. A small population of liver endothelial cells undergoes endothelial-to-mesenchymal transition in response to chronic liver injury. Am J Physiol Gastrointest Liver Physiol 2017;313:G492-G504.
[144] **Ruan B, Duan JL, Xu H**, et al. Capillarized Liver Sinusoidal Endothelial Cells Undergo Partial Endothelial-Mesenchymal Transition to Actively Deposit Sinusoidal ECM in Liver Fibrosis. Front Cell Dev Biol 2021;9:671081.
[145] Abad-Jorda L, Martinez-Alcocer A, Guixe-Muntet S, et al. miR-27b-3p modulates liver sinusoidal endothelium dedifferentiation in chronic liver disease. Hepatol Commun 2025;9.
[146] Gan C, Yaqoob U, Lu J, et al. Liver sinusoidal endothelial cells contribute to portal hypertension through collagen type IV-driven sinusoidal remodeling. JCI Insight 2024;9.
[147] Michalopoulos GK. Liver regeneration. J Cell Physiol 2007;213:286-300.
[148] Janin A, Deschaumes C, Daneshpouy M, et al. CD95 engagement induces disseminated endothelial cell apoptosis in vivo: immunopathologic implications. Blood 2002;99:2940-2947.
[149] **Welz M, Eickhoff S**, Abdullah Z, et al. Perforin inhibition protects from lethal endothelial damage during fulminant viral hepatitis. Nat Commun 2018;9:4805.

[150] **Kojima H, Hirao H**, Kadono K, et al. Cold stress-induced ferroptosis in liver sinusoidal endothelial cells determines liver transplant injury and outcomes. JCI Insight 2024;9.
[151] Wu Y, Li L, Li W, et al. Stretch-induced hepatic endothelial mechanocrine promotes hepatocyte proliferation. Hepatology 2025;82:370-387.
[152] **Ishikawa J, Takeo M**, Iwadate A, et al. Mechanical homeostasis of liver sinusoid is involved in the initiation and termination of liver regeneration. Commun Biol 2021;4:409.
[153] Schoen JM, Wang HH, Minuk GY, et al. Shear stress-induced nitric oxide release triggers the liver regeneration cascade. Nitric Oxide 2001;5:453-464.
[154] Garcia-Trevijano ER, Martinez-Chantar ML, Latasa MU, et al. NO sensitizes rat hepatocytes to proliferation by modifying S-adenosylmethionine levels. Gastroenterology 2002;122:1355-1363.
[155] Hu J, Srivastava K, Wieland M, et al. Endothelial cell-derived angiopoietin-2 controls liver regeneration as a spatiotemporal rheostat. Science 2014;343:416-419.
[156] Shimizu H, Miyazaki M, Wakabayashi Y, et al. Vascular endothelial growth factor secreted by replicating hepatocytes induces sinusoidal endothelial cell proliferation during regeneration after partial hepatectomy in rats. J Hepatol 2001;34:683-689.
[157] Kron P, Linecker M, Limani P, et al. Hypoxia-driven Hif2a coordinates mouse liver regeneration by coupling parenchymal growth to vascular expansion. Hepatology 2016;64:2198-2209.
[158] **Ma X, Huang T, Chen X**, et al. Molecular mechanisms in liver repair and regeneration: from physiology to therapeutics. Signal Transduct Target Ther 2025;10:63.
[159] Elias G, Schonfeld M, Saleh S, et al. Sepsis-induced endothelial dysfunction drives acute-on-chronic liver failure through Angiopoietin-2-HGF-C/EBPbeta pathway. Hepatology 2023;78:803-819.
[160] **Zhang P, Li H**, Zhou C, et al. Single-Cell RNA Transcriptomics Reveals the State of Hepatic Lymphatic Endothelial Cells in Hepatitis B Virus-Related Acute-on-Chronic Liver Failure. J Clin Med 2022;11.
[161] Kou K, Sun X, Tian G, et al. The Mechanisms of Systemic Inflammatory and Immunosuppressive Acute-on-Chronic Liver Failure and Application Prospect of Single-Cell Sequencing. J Immunol Res 2022;2022:5091275.
[162] **Tripathi DM, Vilaseca M**, Lafoz E, et al. Simvastatin Prevents Progression of Acute on Chronic Liver Failure in Rats With Cirrhosis and Portal Hypertension. Gastroenterology 2018;155:1564-1577.
[163] Hirschfield GM, Gershwin ME. The immunobiology and pathophysiology of primary biliary cirrhosis. Annu Rev Pathol 2013;8:303-330.
[164] Babbs C, Haboubi NY, Mellor JM, et al. Endothelial cell transformation in primary biliary cirrhosis: a morphological and biochemical study. Hepatology 1990;11:723-729.
[165] **Zhang P, Li X, Liang J,** et al. Chenodeoxycholic acid modulates cholestatic niche through FXR/Myc/P-selectin axis in liver endothelial cells. Nat Commun 2025;16:2093.
[166] Yang H, Duan Z. Bile Acids and the Potential Role in Primary Biliary Cirrhosis. Digestion 2016;94:145-153.
[167] Xing Y, Zhao T, Gao X, et al. Liver X receptor alpha is essential for the capillarization of liver sinusoidal endothelial cells in liver injury. Sci Rep 2016;6:21309.
[168] **Andrews TS, Nakib D**, Perciani CT, et al. Single-cell, single-nucleus, and spatial transcriptomics characterization of the immunological landscape in the healthy and PSC human liver. J Hepatol 2024;80:730-743.
[169] Yang M, Zhang C. The role of liver sinusoidal endothelial cells in cancer liver metastasis. Am J Cancer Res 2021;11:1845-1860.
[170] Lefere S, Van de Velde F, Hoorens A, et al. Angiopoietin-2 Promotes Pathological Angiogenesis and Is a Therapeutic Target in Murine Nonalcoholic Fatty Liver Disease. Hepatology 2019;69:1087-1104.

[171] Yoshiji H, Noguchi R, Namisaki T, et al. Combination of sorafenib and angiotensin-II receptor blocker attenuates preneoplastic lesion development in a non-diabetic rat model of steatohepatitis. J Gastroenterol 2014;49:1421-1429.
[172] Chen X, Higgins J, Cheung ST, et al. Novel endothelial cell markers in hepatocellular carcinoma. Mod Pathol 2004;17:1198-1210.
[173] **Geraud C, Mogler C**, Runge A, et al. Endothelial transdifferentiation in hepatocellular carcinoma: loss of Stabilin-2 expression in peri-tumourous liver correlates with increased survival. Liver Int 2013;33:1428-1440.
[174] Zhuang PY, Wang JD, Tang ZH, et al. Higher proliferation of peritumoral endothelial cells to IL-6/sIL-6R than tumoral endothelial cells in hepatocellular carcinoma. BMC Cancer 2015;15:830.
[175] Mitsuhashi N, Shimizu H, Ohtsuka M, et al. Angiopoietins and Tie-2 expression in angiogenesis and proliferation of human hepatocellular carcinoma. Hepatology 2003;37:1105-1113.
[176] Thompson KJ, Austin RG, Nazari SS, et al. Altered fatty acid-binding protein 4 (FABP4) expression and function in human and animal models of hepatocellular carcinoma. Liver Int 2018;38:1074-1083.
[177] Laouirem S, Sannier A, Norkowski E, et al. Endothelial fatty liver binding protein 4: a new targetable mediator in hepatocellular carcinoma related to metabolic syndrome. Oncogene 2019;38:3033-3046.
[178] Milner KL, van der Poorten D, Xu A, et al. Adipocyte fatty acid binding protein levels relate to inflammation and fibrosis in nonalcoholic fatty liver disease. Hepatology 2009;49:1926-1934.
[179] Velliou RI, Legaki AI, Nikolakopoulou P, et al. Liver endothelial cells in NAFLD and transition to NASH and HCC. Cell Mol Life Sci 2023;80:314.
[180] Arteta B, Lasuen N, Lopategi A, et al. Colon carcinoma cell interaction with liver sinusoidal endothelium inhibits organ-specific antitumor immunity through interleukin-1-induced mannose receptor in mice. Hepatology 2010;51:2172-2182.
[181] Shetty S, Lalor PF, Adams DH. Liver sinusoidal endothelial cells - gatekeepers of hepatic immunity. Nat Rev Gastroenterol Hepatol 2018;15:555-567.
[182] Sorensen KK, McCourt P, Berg T, et al. The scavenger endothelial cell: a new player in homeostasis and immunity. Am J Physiol Regul Integr Comp Physiol 2012;303:R1217-1230.
[183] **Limmer A, Ohl J, Wingender G,** et al. Cross-presentation of oral antigens by liver sinusoidal endothelial cells leads to CD8 T cell tolerance. Eur J Immunol 2005;35:2970-2981.
[184] **Goubier A, Dubois B**, Gheit H, et al. Plasmacytoid dendritic cells mediate oral tolerance. Immunity 2008;29:464-475.
[185] Knolle PA, Wohlleber D. Immunological functions of liver sinusoidal endothelial cells. Cell Mol Immunol 2016;13:347-353.
[186] Heymann F, Tacke F. Immunology in the liver--from homeostasis to disease. Nat Rev Gastroenterol Hepatol 2016;13:88-110.
[187] Tateya S, Rizzo NO, Handa P, et al. Endothelial NO/cGMP/VASP signaling attenuates Kupffer cell activation and hepatic insulin resistance induced by high-fat feeding. Diabetes 2011;60:2792-2801.
[188] McMahan RH, Porsche CE, Edwards MG, et al. Free Fatty Acids Differentially Downregulate Chemokines in Liver Sinusoidal Endothelial Cells: Insights into Non-Alcoholic Fatty Liver Disease. PLoS One 2016;11:e0159217.
[189] Yao Z, Mates JM, Cheplowitz AM, et al. Blood-Borne Lipopolysaccharide Is Rapidly Eliminated by Liver Sinusoidal Endothelial Cells via High-Density Lipoprotein. J Immunol 2016;197:2390-2399.
[190] Uhrig A, Banafsche R, Kremer M, et al. Development and functional consequences of LPS tolerance in sinusoidal endothelial cells of the liver. J Leukoc Biol 2005;77:626-633.
[191] **Wen Y, Lambrecht J**, Ju C, et al. Hepatic macrophages in liver homeostasis and diseases-diversity, plasticity and therapeutic opportunities. Cell Mol Immunol 2021;18:45-56.

[192] **Berg M, Wingender G**, Djandji D, et al. Cross-presentation of antigens from apoptotic tumor cells by liver sinusoidal endothelial cells leads to tumor-specific CD8+ T cell tolerance. Eur J Immunol 2006;36:2960-2970.
[193] Karrar A, Broome U, Uzunel M, et al. Human liver sinusoidal endothelial cells induce apoptosis in activated T cells: a role in tolerance induction. Gut 2007;56:243-252.
[194] Hochst B, Schildberg FA, Bottcher J, et al. Liver sinusoidal endothelial cells contribute to CD8 T cell tolerance toward circulating carcinoembryonic antigen in mice. Hepatology 2012;56:1924-1933.
[195] **Carambia A, Frenzel C**, Bruns OT, et al. Inhibition of inflammatory CD4 T cell activity by murine liver sinusoidal endothelial cells. J Hepatol 2013;58:112-118.
[196] Knolle PA, Limmer A. Control of immune responses by savenger liver endothelial cells. Swiss Med Wkly 2003;133:501-506.
[197] Geijtenbeek TB, Torensma R, van Vliet SJ, et al. Identification of DC-SIGN, a novel dendritic cell-specific ICAM-3 receptor that supports primary immune responses. Cell 2000;100:575-585.
[198] **Tang L, Yang J**, Liu W, et al. Liver sinusoidal endothelial cell lectin, LSECtin, negatively regulates hepatic T-cell immune response. Gastroenterology 2009;137:1498-1508 e1491-1495.
[199] Martinez-Lopez S, Angel-Gomis E, Gomez-Hurtado I, et al. Cirrhosis-downregulated LSECtin can be retrieved by cytokines, shifts the TLR-induced LSECs secretome and correlates with the hepatic Th response. Liver Int 2024;44:996-1010.
[200] Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol 2013;13:159-175.
[201] Wehr A, Baeck C, Heymann F, et al. Chemokine receptor CXCR6-dependent hepatic NK T Cell accumulation promotes inflammation and liver fibrosis. J Immunol 2013;190:5226-5236.
[202] **Chan CJ, Martinet L**, Gilfillan S, et al. The receptors CD96 and CD226 oppose each other in the regulation of natural killer cell functions. Nat Immunol 2014;15:431-438.
[203] **Papaioannou S, See JX**, Jeong M, et al. Liver sinusoidal endothelial cells orchestrate NK cell recruitment and activation in acute inflammatory liver injury. Cell Rep 2023;42:112836.
[204] Kondo R, Iwakiri Y, Kage M, et al. Endotheliopathy of liver sinusoidal endothelial cells in liver disease. Pathol Int 2023;73:381-393.
[205] **Geissmann F, Cameron TO**, Sidobre S, et al. Intravascular immune surveillance by CXCR6+ NKT cells patrolling liver sinusoids. PLoS Biol 2005;3:e113.
[206] Gibert-Ramos A, Sanfeliu-Redondo D, Aristu-Zabalza P, et al. The Hepatic Sinusoid in Chronic Liver Disease: The Optimal Milieu for Cancer. Cancers (Basel) 2021;13.
[207] Hato T, Zhu AX, Duda DG. Rationally combining anti-VEGF therapy with checkpoint inhibitors in hepatocellular carcinoma. Immunotherapy 2016;8:299-313.
[208] Fang ZQ, Ruan B, Liu JJ, et al. Notch-triggered maladaptation of liver sinusoidal endothelium aggravates nonalcoholic steatohepatitis through endothelial nitric oxide synthase. Hepatology 2022;76:742-758.
[209] Ojha U, Kim S, Rhee CY, et al. Endothelial RUNX3 controls LSEC dysfunction and angiocrine LRG1 signaling to prevent liver fibrosis. Hepatology 2024.
[210] **He S, Luo Y, Ma W,** et al. Endothelial POFUT1 controls injury-induced liver fibrosis by repressing fibrinogen synthesis. J Hepatol 2024;81:135-148.
[211] **Khoury M, Guo Q**, Furuta K, et al. Glycogen synthase kinase 3 activity enhances liver inflammation in MASH. JHEP Rep 2024;6:101073.
[212] Asada S, Kaji K, Nishimura N, et al. Tofogliflozin Delays Portal Hypertension and Hepatic Fibrosis by Inhibiting Sinusoidal Capillarization in Cirrhotic Rats. Cells 2024;13.
[213] Starlinger P, Brunnthaler L, McCabe C, et al. Transcriptomic landscapes of effective and failed liver regeneration in humans. JHEP Rep 2023;5:100683.
[214] **Bosch M, Kallin N**, Donakonda S, et al. A liver immune rheostat regulates CD8 T cell immunity in chronic HBV infection. Nature 2024;631:867-875.

[215] **Talamini L, Picchetti P**, Ferreira LM, et al. Organosilica Cages Target Hepatic Sinusoidal Endothelial Cells Avoiding Macrophage Filtering. ACS Nano 2021;15:9701-9716.
[216] **Zhang C, Gao Y, Du C,** et al. Hepatitis B-Induced IL8 Promotes Hepatocellular Carcinoma Venous Metastasis and Intrahepatic Treg Accumulation. Cancer Res 2021;81:2386-2398.
[217] Herrero A, Benedicto A, Romayor I, et al. Inhibition of COX-2 Impairs Colon Cancer Liver Metastasis through Reduced Stromal Cell Reaction. Biomol Ther (Seoul) 2021;29:342-351.
[218] **Huu Hoang T, Sato-Matsubara M**, Yuasa H, et al. Cancer cells produce liver metastasis via gap formation in sinusoidal endothelial cells through proinflammatory paracrine mechanisms. Sci Adv 2022;8:eabo5525.
[219] **Jauch AS, Wohlfeil SA**, Weller C, et al. Lyve-1 deficiency enhances the hepatic immune microenvironment entailing altered susceptibility to melanoma liver metastasis. Cancer Cell Int 2022;22:398.
[220] Manzi M, Bacigalupo ML, Carabias P, et al. Galectin-1 Controls the Proliferation and Migration of Liver Sinusoidal Endothelial Cells and Their Interaction With Hepatocarcinoma Cells. J Cell Physiol 2016;231:1522-1533.
[221] **Kaur S, Kidambi S, Ortega-Ribera M,** et al. In Vitro Models for the Study of Liver Biology and Diseases: Advances and Limitations. Cell Mol Gastroenterol Hepatol 2023;15:559-571.
[222] **Nevzorova YA, Boyer-Diaz Z**, Cubero FJ, et al. Animal models for liver disease - A practical approach for translational research. J Hepatol 2020;73:423-440.
[223] Yu X, Chen L, Liu J, et al. Immune modulation of liver sinusoidal endothelial cells by melittin nanoparticles suppresses liver metastasis. Nat Commun 2019;10:574.
[224] **Li HY, Gao YX, Wu JC**, et al. Single-cell transcriptome reveals a novel mechanism of C-Kit(+)-liver sinusoidal endothelial cells in NASH. Cell Biosci 2024;14:31.
[225] Saviano A, Henderson NC, Baumert TF. Single-cell genomics and spatial transcriptomics: Discovery of novel cell states and cellular interactions in liver physiology and disease biology. J Hepatol 2020;73:1219-1230.
[226] **Harrison SP, Siller R, Tanaka Y,** et al. Scalable production of tissue-like vascularized liver organoids from human PSCs. Exp Mol Med 2023;55:2005-2024.
[227] **Yoon Y, Gong SC**, Kim MY, et al. Generation of Fibrotic Liver Organoids Using Hepatocytes, Primary Liver Sinusoidal Endothelial Cells, Hepatic Stellate Cells, and Macrophages. Cells 2023;12.
[228] Ortega-Ribera M, Fernandez-Iglesias A, Illa X, et al. Resemblance of the human liver sinusoid in a fluidic device with biomedical and pharmaceutical applications. Biotechnol Bioeng 2018;115:2585-2594.
[229] Zhang LF, Wang XH, Zhang CL, et al. Sequential Nano-Penetrators of Capillarized Liver Sinusoids and Extracellular Matrix Barriers for Liver Fibrosis Therapy. ACS Nano 2022;16:14029-14042.
[230] **Voloshyna T, Holte C**, Pospisil J, et al. PACA nanoparticles target and deliver sildenafil to rejuvenate aged mouse liver sinusoidal endothelial cells. Nanotheranostics 2025;9:155-170.
[231] Hide D, Gil M, Andrade F, et al. Simvastatin-loaded polymeric micelles are more effective and less toxic than conventional statins in a pre-clinical model of advanced chronic liver disease. Nanomedicine 2020;29:102267.
[232] Gil M, Khouri L, Raurell I, et al. Optimization of Statin-Loaded Delivery Nanoparticles for Treating Chronic Liver Diseases by Targeting Liver Sinusoidal Endothelial Cells. Pharmaceutics 2023;15.
[233] Liu W, Liu Y, Zhang L, et al. Nucleic acid spheres for treating capillarisation of liver sinusoidal endothelial cells in liver fibrosis. Nat Commun 2025;16:4517.
[234] **Wang SH, Serr I**, Digigow R, et al. Nanoparticle platform preferentially targeting liver sinusoidal endothelial cells induces tolerance in CD4+ T cell-mediated disease models. Front Immunol 2025;16:1542380.
[235] Wang C, Felli E, Fallowfield JA, et al. Vasomics of the liver. Gut 2025;74:1008-1020.

[236] **Priego-Parra BA, Gallego-Duran R**, Roman-Calleja BM, et al. Advancing precision medicine in metabolic dysfunction-associated steatotic liver disease. Trends Endocrinol Metab 2025.

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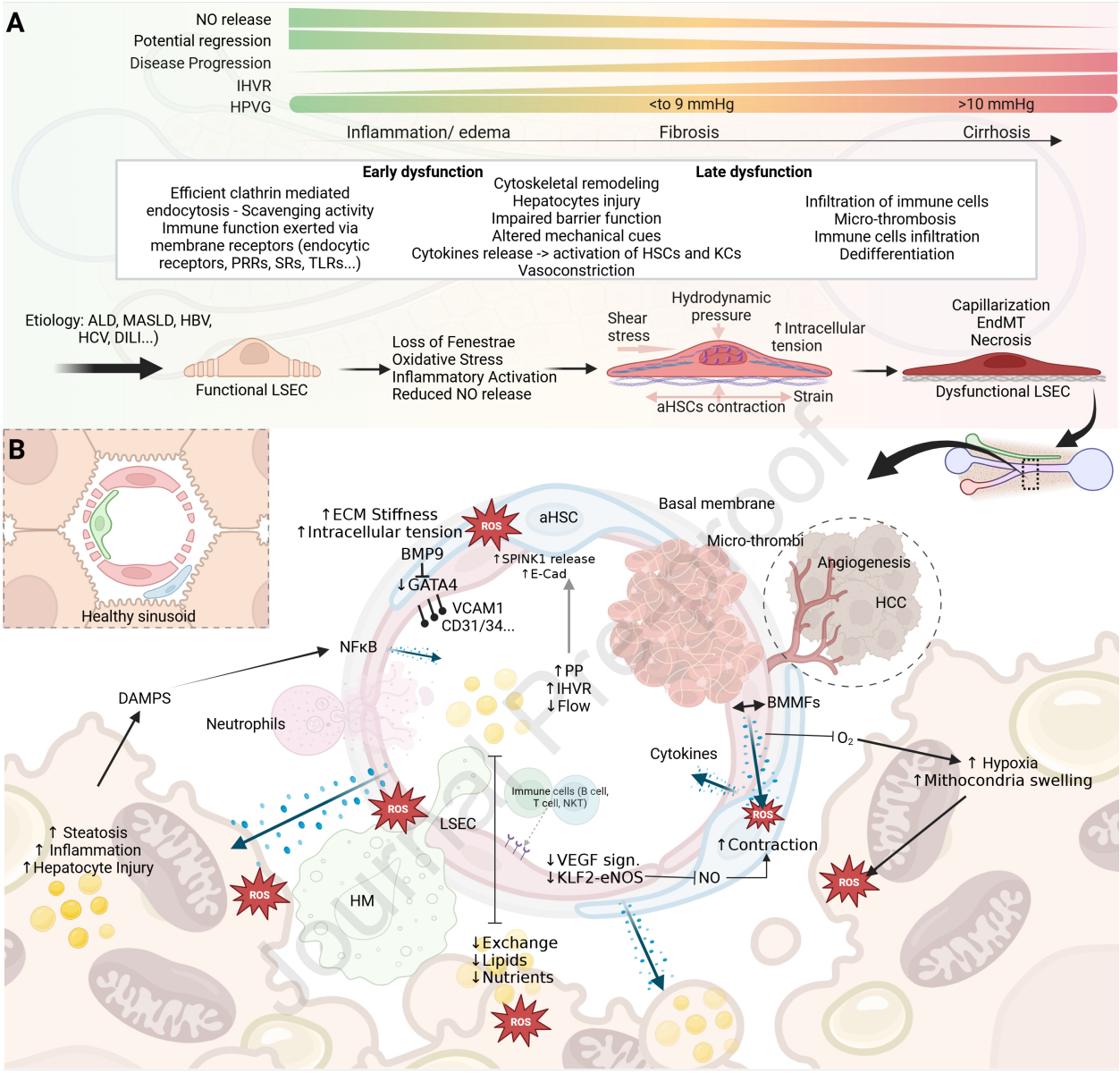
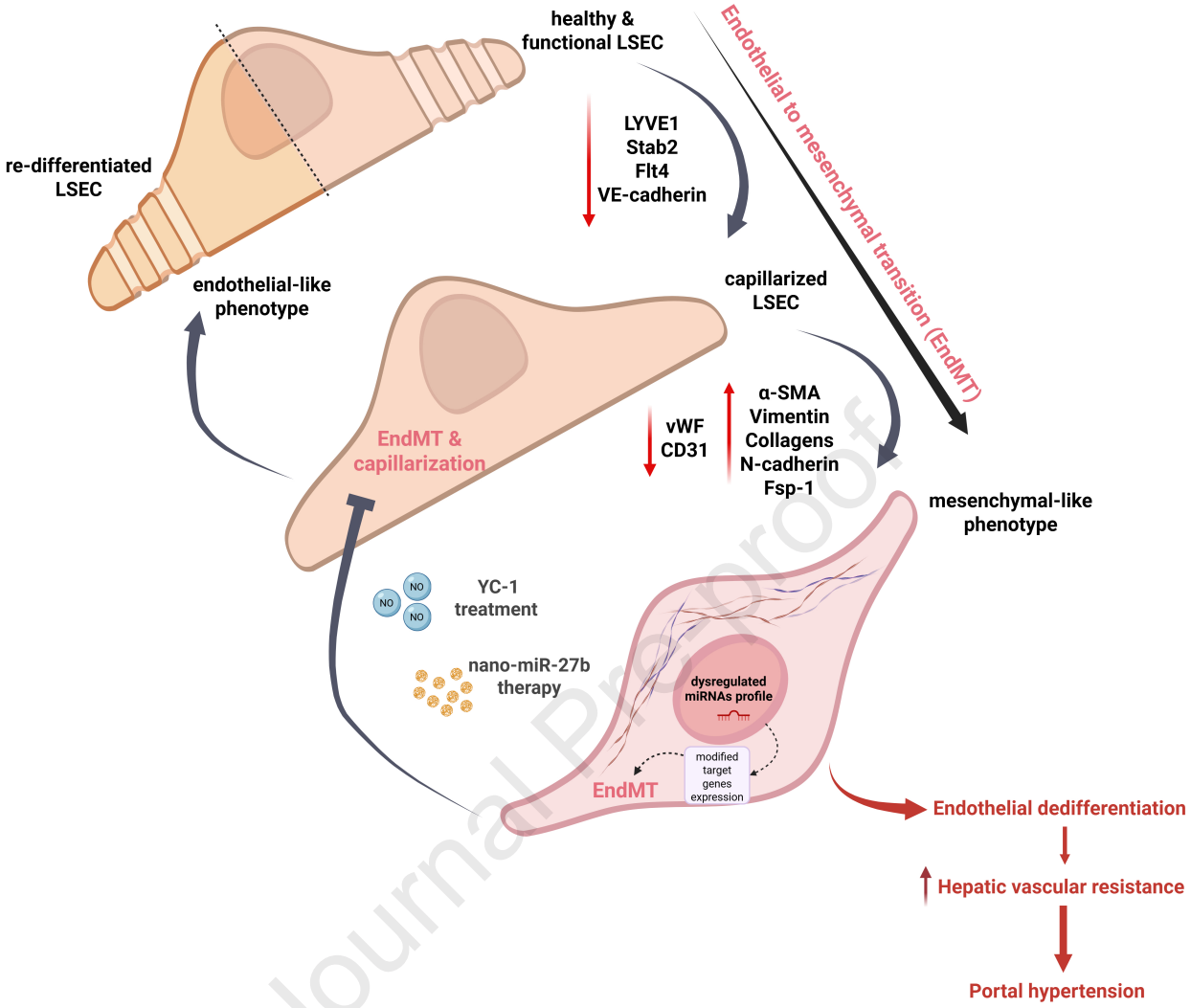

A
NO release
Potential regression
Disease Progression
IHVR
HPVG
<to 9 mmHg
>10 mmHg
Inflammation/ edema
Fibrosis
Cirrhosis
Early dysfunction
Efficient clathrin mediated endocytosis - Scavenging activity
Immune function exerted via membrane receptors (endocytic receptors, PRRs, SRs, TLRs...)
Cytoskeletal remodeling
Hepatocytes injury
Impaired barrier function
Altered mechanical cues
Cytokines release -> activation of HSCs and KCs
Vasoconstriction
Late dysfunction
Infiltration of immune cells
Micro-thrombosis
Immune cells infiltration
Dedifferentiation
Etiology: ALD, MASLD, HBV, HCV, DILI...)
Functional LSEC
Loss of Fenestrae
Oxidative Stress
Inflammatory Activation
Reduced NO release
Shear stress
Hydrodynamic pressure
↑Intracellular tension
aHSCs contraction
Strain
Capillarization
EndMT
Necrosis
Dysfunctional LSEC
B
Healthy sinusoid
↑ECM Stiffness
↑Intracellular tension
ROS
aHSC
BMP9
↓GATA4
VCAM1
CD31/34...
↑SPINK1 release
↑E-Cad
Basal membrane
Micro-thrombi
Angiogenesis
HCC
NFκB
DAMPS
Neutrophils
↑PP
↑IHVR
↓Flow
BMMFs
Cytokines
O2
↑ Hypoxia
↑Mithocondria swelling
Immune cells (B cell, T cell, NKT)
LSEC
↑ Steatosis
↑ Inflammation
↑Hepatocyte Injury
HM
↓VEGF sign.
↓KLF2-eNOS
NO
↑Contraction
↓Exchange
↓Lipids
↓Nutrients



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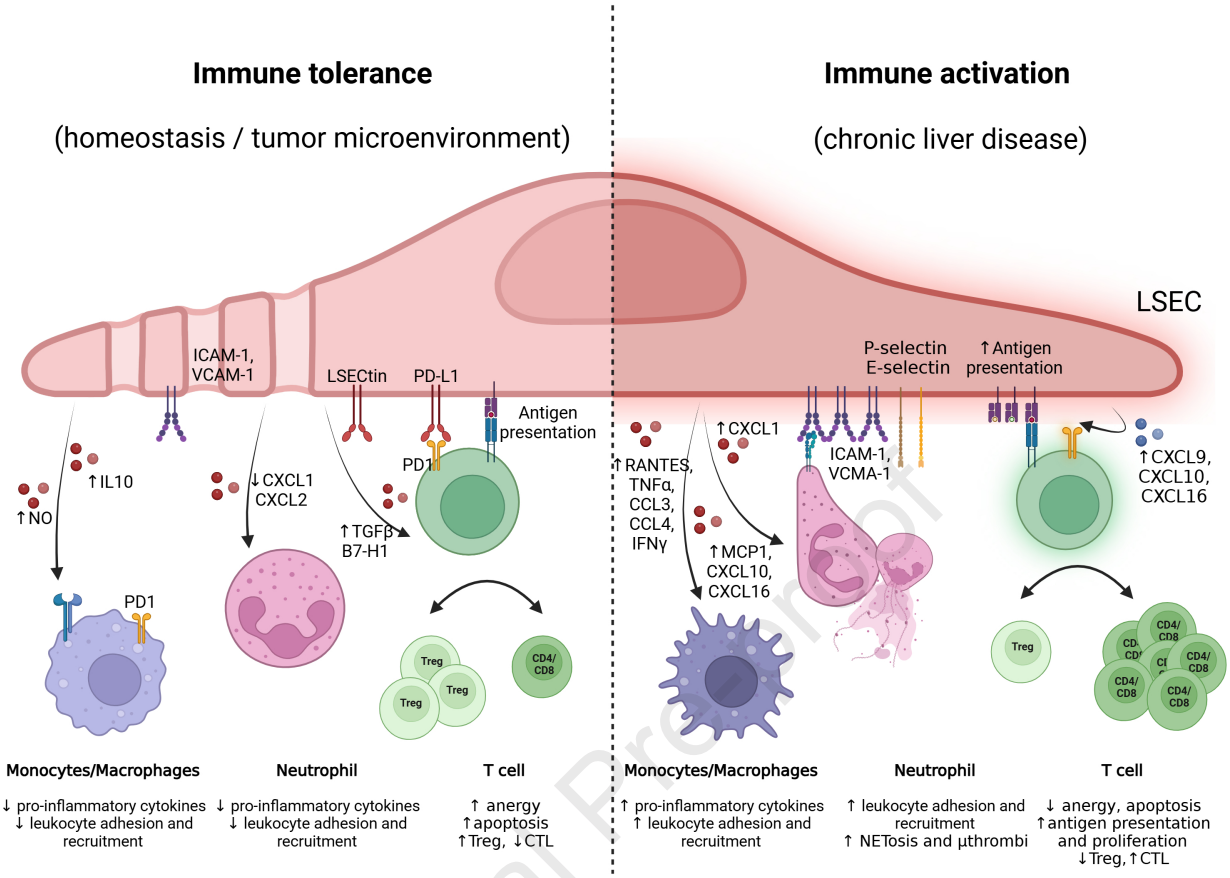

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